Volume I
Lower Willamette River
Reference Area Study
U.S. Army Corps of Engineers
Portland, Oregon

Prepared for
U.S. Army Corps of Engineers

April 9, 2002
7402-01

Lower Willamette River Reference Area Study
U.S. Army Corps of Engineers
Portland, Oregon

Prepared for
U.S. Army Corps of Engineers

April 9, 2002
7402-01

Prepared by
Hart Crowser, Inc.

Taku Fuji, Ph.D.
Associate Toxicologist

Herbert F. Clough, P.E.
Principal

CONTENTS

CONTENTS (Continued)

TABLES

FIGURES

APPENDICES

LOWER WILLAMETTE RIVER REFERENCE AREA STUDY
U.S. ARMY CORPS OF ENGINEERES
PORTLAND, OREGON

1.0 INTRODUCTION

This report presents the results of a study to select and evaluate candidate reference areas within the Lower Willamette River (LWR) system. This reference area study is part of a comprehensive effort by the US Army Corps of Engineers (Corps) to develop a Dredged Material Management Plan (DMMP) for the Willamette River. Reference areas are defined as locations from which reference sediments are obtained to be used as part of a biological testing program. This study was conducted under Contract Number GS-10F-0308K and Order Number DACW57-01-F-0151 for the U.S. Army Corps of Engineers, Portland District.

Hart Crowser completed this study under two phases: a Phase I reconnaissance survey that evaluated 10 potential reference areas by sediment grain size and limited chemical analysis, and a Phase II focused evaluation of three candidate reference areas by comprehensive chemical and biological testing. The results of this study are provided to the Regional Management Team for action.

2.0 PROGRAM OBJECTIVES

The general objective of this study was to identify three reference areas within the LWR with varying grain sizes suitable for use as reference sediment sources for biological testing under Tier III testing requirements of the Regional Dredged Material Evaluation Framework ([DMEF] Corps et al., 1998). Reference sediment is defined in the DMEF as "A whole sediment used to assess sediment conditions that is similar as practicable to the grain size and total organic carbon (TOC) of the dredged material but is free from contamination." Three reference areas were identified that correspond to the established sediment grain size classes for fine-grained (between 70 to 80 percent fines), medium-grained (between 50 to 60 percent fines) and coarse-grained (between 2 and 10 percent fines) sediment found in the in the LWR. Percent fines are defined as the material that passes through the No. 230 sieve (i.e., less than 63 µm) as defined in the DMEF (Corps et al., 1998).

The field sampling program for this reference area study was conducted in two phases: a Phase I reconnaissance survey to evaluate 10 potential reference

areas by grain size, total volatile solids (TVS), and limited chemical analysis; and a Phase II focused evaluation of three candidate reference areas by comprehensive chemical and biological testing (i.e., full suite of DMEF chemicals of concern and biological testing with 10-day lethal and sublethal bioassays using the amphipod *Hyallela azteca* and the midge *Chironomus tentans*). Additionally, Phase II fine-grained and medium-grained sediment were subject to 28-day bioaccumulation testing using the worm *Lumbriculus variegates* and the clam *Corbicula fluminea*. Sampling and testing were performed in a manner consistent with applicable federal and regional guidance documents (Ecology, 1995, EPA/Corps, 1998, and Corps et al., 1998).

3.0 PROJECT TEAM AND RESPONSIBILITIES

The project team assembled for the reference area study provided: (1) project planning and coordination with the Corps Project Manager; (2) chemical and physical analysis of sediment during Phase I and Phase II portions of the study; (3) bioassay and bioaccumulation testing in the Phase II portion of the study; (4) QA/QC management and data validation of analytical results; and (5) interim and final study report preparation. Staffing and responsibilities for these tasks are outlined below.

3.1 Project Planning, Management, and Coordination

Mr. Tim Sherman, Corps, was the representative and the primary contact for administrative and technical issues related to the Corps' involvement with this reference area evaluation project. Dr. Taku Fuji of Hart Crowser's Portland office was the overall project manager responsible for developing and completing the sampling program and for technical issues related to sampling and testing. In addition, Dr. Fuji was responsible for the preparation of the sampling and analysis plan and the interim and final study reports. Mr. Herb Clough of Hart Crowser's Portland office served as the principal in charge for this overall project.

3.2 Field Sampling Program

Dr. Fuji provided overall direction and supervision to the field sampling program in terms of logistics, personnel assignments, and field operations. Mr. Keith Kroeger, Hart Crowser, was the project Field Supervisor, overseeing field collection of surface sediment samples in both phases of fieldwork. Mr. Kroeger was responsible for assuring accurate sample positioning; recording sample locations, depths, and identification; assuring conformance to sampling and handling requirements, including field decontamination procedures;

photographing, describing, and logging the samples; and chain of custody of the samples until they were delivered to the analytical laboratory.

3.3 Chemical and Physical Analyses of Sediments and Tissues

Dr. Fuji was responsible for coordinating the chemical laboratory analyses of sediment and tissues. Mr. Harvey Jackey, Project Chemist at Columbia Analytical Services (CAS) in Kelso, Washington, was responsible for chemical analysis. CAS prepared and analyzed the submitted samples in accordance with DMEF analytical testing protocols and other applicable QA/QC requirements. Mr. Grant Knechtel performed sediment grain size analysis at the Hart Crowser Soils Laboratory in Seattle.

3.4 Bioassay and Bioaccumulation Testing

Dr. Richard Caldwell, Director of Technical Programs, Northwestern Aquatic Sciences (NAS), was responsible for the bioassay and bioaccumulation testing of sediment samples. A written report of the bioassay and bioaccumulation testing results, the protocols used in biological testing, and QA/QC data was prepared by NAS and are included in Appendix A of this final report.

3.5 QA/QC Management

Mr. Howard Cumberland served as Quality Assurance Manager for the sediment assessment and bioassay/bioaccumulation testing program. He performed QA oversight for both the field sampling and laboratory programs. Mr. Neil Morton of Hart Crowser's Seattle office performed the data validation of sediment and tissue analytical data submitted by CAS. The data validation report is included as Appendix B to this report.

3.6 Interim and Final Study Report

There have been three previous documents prepared by Hart Crowser as part of this reference area study:

- Willamette River Reference Area Study, Sampling and Analysis Plan dated August 27, 2001.
- Willamette River Reference Area Study – Phase I Results and Recommendations for Phase II sampling locations dated September 27, 2001.
- Willamette River Reference Area Study – Interim Phase II Results dated November 1, 2001.

This Final Report is a stand alone document incorporating the results and discussions of these previous reports.

4.0 PROJECT AREA DESCRIPTION AND GOALS

4.1 Site Location

The LWR extends from the Willamette's convergence with the Columbia River at river mile (RM) 0 upstream to the Willamette Falls at RM 26.5. The Portland Harbor, where most of the industrial development along the banks of the Willamette River has occurred is the downstream reach from RM 0 to RM 11.6 (Broadway Bridge). All of the proposed reference areas sampled in this study are upriver of the Portland Harbor, extending from RM 15 to RM 25. Figure 1 provides an overview of the study area, and Figures 2 through 5 show the Phase I and Phase II sampling locations along with the target grain size range for each sample.

4.2 Specific Project Goals

The specific goals of the reference area characterization study are summarized below:

- Collect and analyze 10 Phase I surface sediment samples that correspond to the target grain sizes ranges identified as representative of sediment grain sizes found in the LWR in accordance with the protocols and quality assurance/quality control (QA/QC) requirements outlined in the DMEF (i.e., bulk sediment testing);
- Identify three candidate reference areas from the Phase I locations for follow-up Phase II analyses;
- Collect and chemically analyze three surface sediment samples representing the three target grain size ranges for the full suite of DMEF chemicals of concern from the candidate reference areas in accordance with the protocols and QA/QC requirements outlined in the DMEF (i.e., bulk sediment testing);
- Conduct 10-day lethal/sublethal endpoint bioassay testing on the three Phase II surface sediment samples from the candidate reference areas in accordance with available standard protocols (EPA, 2000; ASTM, 1995);
- Conduct bioaccumulation testing using two species on the medium-grained and fine-grained candidate reference area sediment samples in accordance with available standard guidance (EPA, 2000); and

- Report the results of the bulk sediment, grain size, bioassay, and bioaccumulation testing to the Regional Dredge Management Team for action.

5.0 PHASE I SEDIMENT COLLECTION AND HANDLING

The objective of the Phase I reconnaissance survey was to screen potential locations for suitability as reference areas based on sediment grain size characteristics and lack of chemical contamination. Ten surface (0 to 10 centimeters [cm]) sediment samples were proposed to be collected that targeted grain sizes ranges representative of sediment grain sizes found in the LWR.

The 10 reference areas proposed to be sampled included three coarse-grained sediment locations (HC-1, HC-2, and HC-6), three medium-grained sediment locations (HC-3, HC-7, and HC-9), and four fine-grained sediment locations (HC-4, HC-5, HC-8, and HC-10). The grain size targets established for this study were based on an evaluation of Hart Crowser's dredged material characterization studies conducted at various Port of Portland river terminals (Hart Crowser 1997a, 1997 b, 1998, 1999a, and 1999b), additional sediment samples collected from the LWR for site characterization purposes (Hart Crowser 2000a and 2000b), and grain size data from sediment samples collected by the Corps (1996). This evaluation indicated the majority of sediments in the LWR exhibited grain size characteristics that fell within the following three grain size categories: coarse-grained (approximately 2 to 10 percent fines), medium-grained (approximately 50 to 60 percent fines), and fine-grained (approximately 70 to 80 percent fines). In addition, locations were selected from areas thought unlikely to be impacted by known contamination sources (e.g., large municipal discharges) in this reach of the LWR.

5.1 Phase I Sediment Sampling Methods

Surface sediment sampling for Phase I was conducted on August 28, 2001, in accordance with the final Sampling and Analysis Plan (SAP) for this study (Hart Crowser, 2001c). Surface sediment samples for Phase I were collected using a Ponar or van Veen grab sampler. Sediment samples were initially analyzed in the field for sediment grain size distribution using a volumetric wet sieve method. The wet sieving results were used to confirm the target grain size range had been achieved for the proposed Phase I locations. Confirmatory grain size analysis was conducted on Phase I samples in Hart Crowser's soils laboratory. The sediment samples from the Phase I reference areas were also submitted to CAS and analyzed for TVS, total petroleum hydrocarbons (TPH), pesticides, and polychlorinated biphenyls (PCBs).

Specific information on the coordinates of sampling locations and approximate sediment depths (in Columbia River Datum) at the sampling locations are presented in Table 1. Table 2 presents the results of the grain size analysis and material descriptions. Table 3 presents the analytical results of Phase I samples. Laboratory certificates for the analytical chemistry and grain size data are provided in Appendix C to this report.

5.2 Phase I Deviations from the Sampling and Analysis Plan

In Phase I, 10 locations were sampled, though acceptable sediment was collected at only nine of these sampling locations (see discussion below). This study was the first of its kind in the LWR. As such, minor modifications to the SAP were made based on field conditions. The following modifications were made to the protocols provided in Hart Crowser's Final SAP for this project.

- Surface sediment could not be collected from the area designated as HC-6 (Figure 4). HC-6 is located just downriver from Elk Rock Island, and multiple grab sampler deployments in and around the HC-6 sample location could not recover an acceptable sediment sample. Hart Crowser believes the substrate in this area is hard ground, which is consistent with other sediment sampling conducted in this area (Hill and McLaren, 2001). HC-6 was originally targeting coarse-grained sediment. Because three other Phase I samples matched this target grain size range, the inability to collect sediment from HC-6 had no significant affect on program objectives.
- Sediment sample HC-1 was relocated during sampling because the original location (between East Island and Hardtack Island) could not be reached due to low water conditions present in the LWR. The location sampled for HC-1 (Figure 2) was selected as the closest location to the original proposed sampling location that could be sampled. We were able to collect sediment with the grain size target originally proposed, so this modification had no significant affect on program objectives.
- There were slight modifications from proposed sampling locations for several other Phase I sampling locations, as multiple deployments were necessary to collect sediment with the appropriate grain size characteristics, as determined in the field by wet sieving. This was particularly true of the Phase I sediment sampling locations located just upriver from the Sellwood Bridge (HC-3 through HC-5), where small changes in sampling location resulted in significant changes in grain size characteristics of the collected surface sediment (Figure 3). The coordinates of the sampling locations are presented in Table 1.

- The laboratory sediment grain size results that defined percent fines were based on a #200 sieve that calculated percent fines as those particles less than 75 µm rather than 63 µm specified in the SAP (Laboratory Certificates are provided in Appendix C). Subsequent grain size analysis on Phase II sediment samples was conducted using the 63 µm sieve size for differentiating percent fines.
- The grain size target for Sample HC-5 was originally proposed to be fine-grained material based on previous work conducted in the LWR (Hill and McLaren, 2001). However, based on multiple deployments and field wet sieving results, no fine-grained sediment was found at this location. Instead, a medium-grained sediment sample was collected at HC-5 (Table 2).
- The grain size target for Sample HC-7 was originally proposed to be medium-grained material based on previous work conducted in the LWR (Hill and McLaren, 2001). However, based on multiple deployments and field wet sieving results, no medium-grained sediment was found at this location. A coarse-grained sediment sample was collected at HC-7.

6.0 PHASE I RESULTS

6.1 Grain Size Analysis Results

The results of the field wet sieving and laboratory sieve analysis for grain size are presented in Table 2. Wet sieving was used in the field to estimate grain size characteristics of the collected surface sediments to assess if we had successfully met the proposed target ranges. Confirmatory grain size analysis was conducted on all sediment samples by Hart Crowser's soils laboratory using ASTM D 422 (modified, sieve analysis only) methodology. As shown in Table 2, the wet sieving results approximated laboratory-derived sediment grain size results, particularly with respect to the target sediment grain size categories established for this project.

Based on field wet sieving results, Hart Crowser had assumed the sediment collected from within Cedar Island Cove (HC-10) was fine-grained material (greater than 70 percent fines); however, the laboratory grain size data indicated this sediment is more appropriately classified as medium-grained (59.7 percent fines). Conversely, Sample HC-8 collected from within Elk Rock Island matched the target grain size range for medium-grained sediment based on wet sieve results (53 percent fines), but the laboratory grain size data indicates this material is finer grained than originally assumed (64.9 percent fines).

The results of the Phase I sediment grain size analysis showed that none of the sediment samples contained greater than 70 percent fines, which was the original target for fine-grained sediment. However, based on discussion with the Corps, it was agreed that sediment samples with greater than 60 percent fines would be used to represent this grain size category (meeting with Corps on September 11, 2001).

Based on the laboratory grain size data presented in Table 2, the Phase I sediment samples have been divided into the following grain size categories:

Fine-Grained Samples: HC-3 (60.9 percent fines); HC-4 (69.3 percent fines); and HC-8 (64.9 percent fines).

Medium-Grained Samples: HC-5 (25.1 percent fines); HC-9 (43.4 percent fines) and HC-10 (59.7 percent fines).

Coarse-Grained Samples: HC-1 (5 percent fines); HC-2 (9.8 percent fines); and HC-7 (4 percent fines).

6.2 Analytical Chemistry Results for Phase I Samples

Table 3 presents the analytical results for the Phase I sediment samples. There were no detected concentrations of pesticides or PCBs in any of the nine sediment samples collected. For the TPH analysis, there were no detected concentrations of gasoline range organics in any of the Phase I sediment samples. Diesel range organics were detected in four samples (HC-3, HC-4, HC-8, and HC-10), with detected concentrations ranging from 34 mg/kg to 56 mg/kg. Residual range organics were detected in six samples (HC-3, HC-4, HC-5, HC-8, HC-9, and HC-10), with detected concentrations ranging from 72 mg/kg to 290 mg/kg. All of the detections of TPH in Phase I sediment samples were qualified with a "Z" flag by the analytical laboratory. The "Z" qualifier indicates the chromatographic fingerprints from these samples do not resemble a petroleum product. The analytical laboratory believes these detections are most likely non-polar biogenic oils and are not indicative of petroleum-related contamination (pers. comm. Mr. Harvey Jackey, Columbia Analytical Services; September 7, 2001).

6.3 Phase II Sample Location Recommendations

The results of the Phase I sediment sampling were used to recommend three reference areas for follow-up comprehensive chemical and biological testing (Phase II Testing). To select candidate Phase II sampling locations, Hart Crowser developed a decision matrix based on the three characteristics identified in the SAP to prioritize Phase II sample locations (Table 4). Candidate reference areas

selected for further analysis in Phase II were those shown to be substantially free of anthropogenic contaminants, those believed to be stable to permit long-term utilization (i.e., no appreciable erosion or accretion), and those that matched the target grain size characteristics identified in the SAP.

For the decision matrix presented in Table 4, the sediment samples from each of the three target grain size ranges were evaluated with regards to each of the three characteristics and given a subjective score (based on a scale of 1 through 5, with 5 indicating the highest match with program objectives). For example, for the analytical chemistry characteristic, all sediment samples received the highest score of 5 because none had exhibited any anthropogenic contamination. For the stability characteristic, the samples collected from coves or other quiescent areas of the LWR received a higher score than samples collected within or adjacent to the main channel of the river. Finally, for the grain size characteristic, the scores were based on how close the Phase I sediment samples matched the target grain size range.

Therefore, based on the results of the Phase I sampling and the decision matrix evaluation of the Phase I sampling locations, the following sampling locations were recommended for Phase II sampling and analysis:

Fine-Grained Sample: Sample Location HC-8 by Elk Rock Island (Figure 4).

Medium-Grained Sample: Sample Location HC-10 in Cedar Island Cove (Figure 5).

Coarse-Grained Sample: Sample Location HC-2 by Ross Island (Figure 2). All three Phase I coarse-grained sediment sampling locations received an identical score on the decision matrix (Table 4). HC-2 was selected because its location allowed for easier sampling during the low water conditions occurring in the LWR. Location HC-7 was not selected because underwater obstructions entangled the grab sampler during Phase I sampling, and such obstructions could hamper long-term utilization of this location.

These recommendations were presented to and approved by the Corps at a meeting on September 11, 2001.

7.0 PHASE II SEDIMENT COLLECTION AND ANALYSIS

The objective of the Phase II portion of the reference area study was to conduct comprehensive chemical and biological testing on the most promising reference areas identified during the Phase I reconnaissance portion of this study. Three locations were sampled for the Phase II portion of this study: HC-2 representing

coarse-grained sediment reference area, HC-10 representing the medium-grained sediment reference area, and HC-8 representing the fine grained sediment reference area.

Sediment sampling and analysis for the Phase II sampling was conducted on September 17, 2001, in accordance with the SAP for this study (Hart Crowser, 2001c). Sediments were collected using an air powered van Veen "grab" type sampler as discussed in the SAP. Specific information on the coordinates of sampling locations and approximate sediment depths (in Columbia River Datum) at the sampling locations are presented in Table 5. Table 6 presents the results of the grain size, TOC, and TVS analysis and material descriptions. Table 7 presents the analytical chemistry results of the three Phase II samples. Tables 8 through 10 present the results of the amphipod (*Hyalella azteca*) 10-day survival bioassay and the 10-day midge (*Chironomus tentans*) survival and growth bioassays. Laboratory certificates for the analytical chemistry and grain size data are provided in Appendix C of this report. Bioassay and bioaccumulation testing reports are provided in Appendix A of this report. The tissue results from the 28-day bioaccumulation test with the worm (Lumbriculus variegates) are presented in Table 11, and the tissue results from the 28-day bioaccumulation test with the clam (*Corbicula fluminea*) are presented in Table 12.

7.1 Phase II Modifications to the Sampling and Analysis Plan

The three potential reference areas were sampled, and acceptable sediment was collected at all of these sampling locations (see Figures 2, 3, and 5). All sampling and analysis was conducted in accordance with the SAP, with the following modifications.

- Bioaccumulation testing of the medium-grained (HC-10) and fine-grained (HC-8) sediment locations required the collection of a large volume (70 liters) of sediment at these sites. The sediments at these locations were collected from the top 12 inches of sediment, as opposed to the top 4 inches, to assist in sample volume collection. No stratification of the surface sediment was observed in these samples over this depth.
- At the request of the Corps, Hart Crowser collected five additional sediment samples from Cedar Island Cove, samples CD-1 through CD-5, for sediment grain size, TOC, and TVS analysis. The objective of these samples was to further characterize the range of grain sizes available at this location.

8.0 PHASE II RESULTS

8.1 Grain Size Analysis Results

The results of the grain size testing of Phase II samples are presented in Table 6. Grain size analysis was conducted on all sediment samples by Hart Crowser's soils laboratory using ASTM D 422 (modified, hydrometer analysis) methodology. The grain size results indicate that samples HC-2 (coarse-grained) and HC-10 (medium-grained) achieved the target ranges proposed in the SAP. The target range for coarse-grained sediment was to collect sediment with grain size between 2 to 10 percent fines, and for medium-grained sediment, the target range was between 50 to 60 percent fines. The grain size of HC-2 was 6.1 percent fines and HC-10 was 52.8 percent fines. The target range for fine-grained sediment was established as between 70 to 80 percent fines, and the grain size of HC-8 was slightly below this target range (66.3 percent fines); however, this grain size is close to the target range, and the use of this grain size class was approved by the Corps. The use of this sediment as a Phase II sample had no impact on program objectives.

The results of the additional samples collected at Cedar Island Cove (Figure 5) indicate there is a range of sediment grain sizes at this location, ranging from 21.4 percent fines to 89.9 percent fines that can be used for subsequent studies, if necessary. Based on field observations, it is also likely that Elk Rock Island State Park contains a range of sediment grain sizes that can be used for subsequent studies.

8.2 Sediment Analytical Chemistry Results

The analytical chemistry results for the three Phase II sediment samples are presented in Table 7 along with the corresponding DMEF Screening Levels (SLs; Corps et al., 1998). For Phase II Sample HC-2 (coarse-grained sediment), there were no constituents detected above DMEF SLs. There were several polycyclic aromatic hydrocarbons (PAHs) detected in this sample; however, all but one of these detections were "J" flagged by the analytical laboratory, indicating these are estimated values, as the detected levels are between the method reporting limit (MRL) and the method detection limit (MDL). Only benzo(g,h,i)perylene was detected at a concentration above the MRL. The pesticide 4,4'-DDT was detected at a low concentration (0.73 µg/kg), which was below the MRL and is an estimated value. No other semivolatile organic compounds (SVOC), pesticides, or PCBs were detected in this sample. The concentrations of metals detected in Sample HC-2 were low and likely represent regional background levels. The concentration of tributyltin (TBT) in sediment pore water was

reported to be 0.07 µg/L, which is below the DMEF SL of 0.15 µg/L (Corps, et al., 1998).

For Phase II Sample HC-8 (fine-grained sediment), there were no constituents detected above DMEF SLs. Again, there were several PAHs detected at low concentrations in this sample; however, all of these detections were "J" flagged by the analytical laboratory, indicating these are estimated values, as the detected levels are between the MRL and the MDL. Other organics detected in this sample were di-n-butyl phthalate, 4-methylphenol, and benzoic acid; however, all were detected at concentrations below their corresponding MRL. The pesticide 4,4'-DDT and its metabolites 4,4'-DDE and 4,4'-DDD were detected at low levels in this sample. All were below their corresponding MRL, and the total DDT (sum of 4,4'-DDT and its metabolites) concentration of 3.15 µg/kg was below the total DDT DMEF SL of 6.9 µg/kg. No other SVOCs, pesticides, or PCBs were detected in this sample. The concentrations of metals detected in Sample HC-8 were low and likely represent regional background levels. The concentration of TBT in sediment pore water was reported to be 0.07 µg/L, which is below the DMEF SL of 0.15 µg/L.

For Phase II Sample HC-10, (medium-grained sediment), total DDT was the only constituent detected above DMEF SLs. There were several PAHs detected in this sample; however, the majority of these detections were "J" flagged by the analytical laboratory, and only pyrene and flouranthene were detected at a concentration above their corresponding MRL. Other organics detected in this sample were phenol, 4-methylphenol, benzoic acid, and di-n-butyl phthalate; however, all were detected at concentrations below their corresponding MRL. The pesticide 4,4'-DDT and its metabolites 4,4'-DDE and 4,4'-DDD were detected in this sample. Total DDT (sum of 4,4'-DDT and its metabolites) in Sample HC-10 was 14.57 µg/kg, which exceeds the total DDT DMEF SL of 6.9 µg/kg; however, this concentration is below the DMEF total DDT Bioaccumulation Trigger (BT) concentration of 50 µg/kg. No other SVOCs, pesticides, or PCBs were detected in this sample. The concentrations of metals detected in Sample HC-10 were low and likely represent regional background levels. The concentration of TBT in sediment pore water was reported to be 0.064 µg/L, which is below the DMEF SL of 0.15 µg/L.

The detection of 4,4'-DDT and its metabolites in the Phase II samples was unexpected. The results of the Phase I sampling indicated that 4,4'-DDT and its metabolites were not present at these sample locations (Hart Crowser, 2001d). We have verified with the analytical laboratory that no QA/QC problems exist associated with either the Phase I or Phase II pesticide analysis.

One difference in sampling methods between Phase I and Phase II for samples HC-8 and HC-10 was that sediment was collected from the top 12 inches for the Phase II samples because of the large volume of sediment required for bioaccumulation testing. Surface sediments for Phase I were collected from the top 10 cm (approximately 4 inches). If DDT is more prevalent in the subsurface sediments in the vicinity of Elk Rock Island (HC-8) and Cedar Island Cove (HC-10), we may not have sampled this depth interval during Phase I sampling, but may have collected this material during Phase II sampling; however, this does not explain the detected concentrations of DDT in Sample HC-2 that was sampled in the same manner (i.e., the top 10 cm) during Phase II as Phase I. Additional sampling may help explain the sources of 4,4'-DDT and its metabolites in these areas.

The detected concentrations of TBT in pore water samples from all three sediment samples were also unexpected. The fact that the pore water concentrations of TBT were similar between these very different sediment samples that have no known source of TBT (HC-2 0.07 ug/L, HC-8 0.07 ug/L, and HC-10 0.064 ug/L) suggests a systematic error. However, Columbia Analytical Services could find no QA/QC problems associated with these analyses. Unfortunately, bulk sediment analysis of TBT in these sediments was not conducted to confirm the presence of TBT. It is recommended that in the future, both bulk sediment and pore water measurements of TBT be conducted to confirm the presence of this compound in the test material

8.3 Bioassay Results

For the Phase II sediment samples, 10-day amphipod and midge lethal and sublethal endopoint bioassays were conducted in accordance with DMEF protocols (Corps, et al., 1998). All three Phase II sediment samples achieved the performance standards established for reference sediments in the DMEF. There is no evidence that exposure to any of these three sediments had any adverse effect on amphipod survival or midge growth and survival in these bioassays. All three of these sediments are acceptable for use as reference sediments when conducting future Tier III biological testing under the DMEF.

Table 8 presents the results of the 10-day amphipod survival bioassay using *Hyalella azteca* along with the performance criteria established for control and reference sediments for this bioassay under the DMEF (Corps et al., 1998). The mean test mortality in the three Phase II sediments had similar mean mortalities as the negative control. The mean mortalities for sediment HC-8 and HC-10 were 6.3 percent, for sediment HC-2 the mean mortality was 7.5 percent, and the mean mortality for the negative control was 6.3 percent. The performance criteria were met for each of the Phase II sediments and also for the negative

control. Therefore, these tests are considered valid bioassays, and each of the Phase II sediments performed at a level required for reference sediments under the DMEF for this bioassay.

Table 9 presents the results of the 10-day midge survival bioassay using *Chironomus tentans* along with the performance criteria established for control and reference sediments for this bioassay under the DMEF (Corps et al., 1998). The mean test mortalities in the three Phase II sediments (13.8, 7.5, and 16.3 percent) were all less than the mean mortalities of the negative control (18.8 percent). The performance criteria were met for each of the Phase II sediments and also for the negative control. Therefore, these tests are considered valid bioassays, and each of the Phase II sediments performed at a level required for reference sediments under the DMEF for this bioassay.

Table 10 presents the results of the 10-day midge growth bioassay using *Chironomus tentans* along with the performance criteria established for control and reference sediments for this bioassay under the DMEF (Corps et al., 1998). The mean test individual biomass (a measure of organism growth at the termination of the bioassay) in the three Phase II sediments was greater than the mean individual biomass of the negative control. Therefore, the growth in the three Phase II samples exceeded the growth found in the negative control. The performance criteria were met for each of the Phase II sediments and also for the negative control. Therefore, these tests are considered valid bioassays, and each of the Phase II sediments performed at a level required for reference sediments under the DMEF for this bioassay.

8.4 Bioaccumulation Testing Program

For this project, bioaccumulation testing was only proposed for the medium-grained and fine-grained candidate reference sediments (Hart Crowser, 2001c). Two species of benthic organisms were used for bioaccumulation testing, the oligochaete *Lumbriculus variegates* and the bivalve *Corbicula fluminea*. The 28-day bioaccumulation tests were conducted in general accordance with available guidance (EPA/Corps, 1998 and EPA, 2000). An optional schedule of overlying water renewal (three times per week) based on the "Inland Testing Manual" (EPA/Corps, 1998) was undertaken for both tests. It should be noted that no standard method currently exists for the 28-day *Corbicula fluminea* test, and the protocol followed by NAS is included in Appendix A.

Lumbriculus variegates is the standard freshwater bioaccumulation testing organism but has limited tissue biomass available for analytical chemistry testing of tissues, which limits the types of chemical analysis that can be conducted on tissues at the conclusion of the standard 28-day laboratory bioaccumulation test.

Therefore, for this project, the bivalve *Corbicula fluminea* was also tested, as these are larger organisms and provide greater tissue mass for chemical analysis.

The *Lumbriculs variegates* bioaccumulation testing of sediments HC-8 and HC-10 was conducted according to schedule with no problems. The test was initiated on October 2, 2001, and completed on October 30, 2001. No QA/QC problems were encountered with the *Lumbriculs variegates* bioaccumulation tests, and these tests are considered acceptable for use.

The Time Zero (Test Initiation) and Day 28 (Test Completion) tissue samples were delivered to CAS on October 16 and November 7, 2001, respectively. The limited tissue mass available for Day 28 (Test Completion) *Lumbriculus variegates* samples (average sample wet weight per replicate was approximately 8.5 grams) made it impossible to analyze these tissues for all classes of chemical constituents. Because TBT and DDT (and its metabolites) were detected in the HC-8 and HC-10 sediment samples, it was decided the tissue samples would only be analyzed for percent lipids, TBT, and pesticides/PCBs. The results of the tissue analytical chemistry results for *Lumbriculus variegates* are presented in Table 11.

For the *Corbicuula fluminea*, there were difficulties encountered in obtaining healthy populations of organisms for the bioaccumulation tests. The primary source of *Corbicula fluminea* has been Brezina & Associates, located in Dillon Beach, CA. Brezina and Associates collect *Corbicula fluminea* from the Sacramento River Delta between Antioch and Pittsburg, California. One thousand (1,000) *Corbicula fluminea* were collected between September 20 and 21, 2001. By the following morning, 40 to 50 animals had died and continued to die at that rate and higher (to approximately 100 per day) over the course of the one-week holding time (pers. comm. with Mr. Gerald Irisarri of NAS on September 28, 2001). A second batch of *Corbicula fluminea* was collected on October 4 and 5, 2001, with the same result. John Bezina speculated the high river temperatures may have stressed the animals and may have induced spawning, further weakening the animals.

A second source of *Corbicula fluminea*, from TAI Environmental Sciences of Mobile Alabama, was located. TAI collects their *Corbicula fluminea* from the Cahaba River near Birmingham, Alabama. Because NAS (the bioassay laboratory) had not used animals from this source before in biological testing, quick turn-around tissue analysis for metals and pesticides/PCBs was done on a composite sample of *Corbicula fluminea* tissue collected from the Cahaba River to ensure these animals were of sufficient quality for use in bioaccumulation tests. The chemical analysis did not detect any pesticides or PCBs in the *Corbicula fluminea* tissue, and the levels of metals reported were similar to levels

reported by Brezina and Associates for *Corbicula fluminea* collected from the Sacramento River. Based on the tissue chemistry results and the fact the animals sent by TAI were healthy after one week of holding at the NAS laboratory, Hart Crowser decided to go forward with *Corbicula fluminea* bioaccumulation testing using the organisms from the Cahaba River. The *Corbicula fluminea* bioaccumulation tests were initiated on October 29, 2001, which was well within the eight-week sediment holding time allowed for bioaccumulation testing under the DMEF (Corps, et al., 1998).

The bioaccumulation test with *Corbicula fluminea* was completed on November 26, 2001. The *Corbicula fluminea* tissue samples from Time Zero and Day 28 were delivered to CAS on November 29, 2001. The tissue mass available for Day 28 (Test Completion) *Corbicula fluminea* samples (average wet weight per replicate was approximately 35.2 grams for sediment HC-8 exposure and 26.2 grams for sediment HC-10 exposure) allowed for the full suite of DMEF chemicals of concern to be analyzed in *Corbicula fluminea* tissues. The results of the tissue analytical results for *Corbicula fluminea* are presented in Table 12. One replicate sample from Day 28 sediment HC-8 exposure (replicate CAS lab ID K2108883-006) was lost during processing of organic extracts by CAS, and no additional tissue was available for repeating the extraction. Therefore, only lipid, total solids, metals, and butyltin results are available for that replicate sample, and only four replicate data points are available for the organic analytical chemistry results for this treatment. No QA/QC problems were encountered with the *Corbicula fluminea* bioaccumulation test, and these tests are considered acceptable for use.

8.5 Bioaccumulation Testing Tissue Analytical Chemistry Results

The results of the 28-day laboratory bioaccumulation tissue results for both *Lumbriculus variegates* and *Corbicula fluminea* are discussed in further detail in the following sections. Emphasis is placed on the evaluation of tissue results of TBT and DDT and its metabolites DDD and DDE, as these were the bioaccumulative compounds detected at low levels in the proposed reference sediments collected at Elk Rock Island (HC-8) and Cedar Island Cove (HC-10).

8.5.1 *Lumbriculus variegates* Bioaccumulation Tissue Analysis Results

The analytical chemistry results for the Time Zero and Day 28 tissue residue concentrations from exposure to sediment samples HC-8 and HC-10 are presented in Table 11. Table 11 presents the individual replicate results (five replicates per treatment) and also the mean tissue concentration for each treatment. Mean tissue concentration were calculated using one-half of the

MDL for undetected replicates within a treatment. The MDLs for individual replicate tissue samples are presented in the laboratory certificates in Appendix D. As discussed previously, because of the low sample masses available at the termination of the tests, only limited chemical analysis was performed on the Day 28 tissue samples. The analysis that was completed included percent lipids, butyltins, and pesticides/PCBs. For the Time Zero samples collected at test initiation, there were no limitations based on sample mass, and the full suite of DMEF chemicals of concern was analyzed in these tissues.

Lipid Results. The percent lipids reported in the Time Zero samples (mean = 1.17 percent) are similar to those reported in both the 28 day HC-8 exposure treatment (mean = 1.02 percent) and HC-10 exposure treatment (mean = 0.98 percent) indicating that the organisms remained healthy throughout the duration of the test. It should be noted the organisms are not fed during the 28-days of exposure and must rely on organic matter present in the test sediments for nutrition.

Butyltin Results. The butyltin results for *Lumbriculus variegates* were unexpected. As shown in Table 11, TBT was not detected in any of the treatments, but both di-n-butyltin and n-butyltin were detected in all replicate samples from the three treatments. This is a very unusual result as TBT is almost always present when the other butyltin compounds are present in tissue (pers. comm. with Ms. Abbie Spielman of CAS, February 5, 2002). Additionally, TBT was reported to be present in the porewater of test sediments HC-8 and HC-10 at concentrations of 0.07 µg/L and 0.067 µg/l, respectively. As TBT was not detected in tissue from either of the Day 28 treatments, these results indicate TBT at these levels did not result in any detectable accumulation in *Lumbriculus variegates* over the exposure period. Hart Crowser asked both CAS and NAS to review their files and procedures to assess potential sources for these butyltin results, and the results of this assessment are discussed in the uncertainty section below.

DDT Results. For the pesticide DDT and its metabolites DDE and DDD, very low levels were detected in *Lumbriculus variegates* tissue in the Time Zero samples. The majority of these data were J-flagged by the laboratory, indicating the concentrations are estimated as the reported concentration is below the MRL but above or equal to the MDL and that there is uncertainty in these results. The mean concentration of Time Zero tissue samples for total DDT (sum of DDT and its metabolites) was 3.14 µg/kg wet weight. The tissue results from Day-28 for exposure to sample HC-8 reported a mean total DDT concentration of 2.0 µg/kg wet weight; all of these data were J-flagged by the laboratory and are estimated concentrations. The tissue results from Day-28 for exposure to sample HC-10 reported a mean total DDT concentration of 4.8 µg/kg wet

weight. The total DDT concentrations reported for the Day 28 tissue samples for both test sediment treatments are based on the reported concentrations of the DDT metabolites, DDE and DDD. The parent compound DDT, was not detected in these tissue samples. The analytical results from these test sediments (Table 7) report a total DDT concentration 3.15 µg/kg (J-flagged) in sample HC-8 and 14.57 µg/kg in sample HC-10.

To determine whether there was a statistically significant difference between the reported concentrations of total DDT in the Time Zero treatment and the Day 28 treatments for exposure to sediments HC-8 and HC-10, a paired one-tailed student's t-test was performed with a p-value of 0.05. The t-tests were performed using both wet weight concentrations and lipid-normalized values. As the percent lipid was similar between all treatments, there was no difference in the results of the statistical testing between these two methods of evaluating the tissue data. The t-test results for total DDT tissue mean concentrations indicated there was a statistically significant difference between tissue levels between Time Zero and Time 28 day exposures in Sample HC-10 for *Lumbriculus variegates*. Conversely, there was no significant difference in sample tissue means between Time Zero and Time 28 day exposure in Sample HC-08 for *Lumbriculus variegates*.

These data indicate the level of DDT (and its metabolites) reported for test sediment HC-10 (14.57 µg/kg) resulted in significantly increased tissue concentration in *Lumbriculus variegates* over the 28-day exposure period. However, an evaluation of the toxicological significance of the reported tissue residues was beyond the scope of this present study. Additionally, these data indicate the level of DDT (and its metabolites) detected in test sediment sample HC-8 (3.15 µg/kg) did not result in any detectable accumulation in *Lumbriculus variegates* over the exposure period.

8.5.2 *Corbicula fluminea* Bioaccumulation Tissue Analysis Results

The analytical chemistry results for the Time Zero and Day 28 tissue residue concentrations from *Corbicula fluminea* exposure to sediment samples HC-8 and HC-10 are presented in Table 12. Mean tissue concentration were calculated using one-half of the MDL for undetected replicates within a treatment. Similar to the discussion of *Lumbriculus variegates* tissue residue results, this section will focus on the results of the TBT and pesticides results. Additionally, the results of the metal and other organic compounds analyzed in *Corbicula fluminea* tissue will be discussed.

Lipid Results. The percent lipids reported in the Time Zero samples (mean = 0.86 percent) are similar to those reported in both the 28 day HC-8 exposure

treatment (mean = 0.75 percent) and HC-10 exposure treatment (mean = 0.92 percent) indicating that the organisms remained healthy throughout the duration of the test.

Butyltin Results. In contrast to the *Lumbriculus variegates*, there was TBT (as well as di-n-butyltin and n-butytin) detected in all replicate samples from the three treatments. Again, because TBT was reported in porewater in test sediments HC-8 and HC-10, a paired one-tailed student's t-test was performed to determine whether there was a statistically significant difference (p = 0.05) in the reported mean concentrations of tissue TBT between the Time Zero treatment and the test sediment results. The t-tests showed there were no significant differences between Time Zero and Time 28 day tissue residues in Samples HC-08 or HC-10 for *Corbicula fluminea*. In fact, the mean concentrations for TBT decreased between the Time Zero samples and the Day 28 treatment samples for both test sediment samples as shown in Table 12. These results indicate TBT at the levels reported in test sediment porewater did not result in any detectable accumulation in *Corbicula fluminea* over the 28-day exposure period.

DDT Results. DDE was reported at very low levels in *Corbicula fluminea* tissue in the Time Zero samples. All of these data were J-flagged by the laboratory, indicating the concentrations are estimated as the reported concentration is below the MRL but above or equal to the MDL and that there is uncertainty in these results. Neither DDT nor DDD were detected in the Time Zero tissue samples. The mean concentration of Time Zero tissue samples for DDE was 0.68 µg/kg wet weight. The tissue results from Day-28 for exposure to sample HC-8 reported a DDE concentration of 1.2 µg/kg wet weight; three of the replicate samples were J-flagged and one was not detected at a MDL of 1.9 µg/kg. The tissue results from Day-28 for exposure to sample HC-10 reported a mean DDE concentration of 1.4 µg/kg wet weight with three of the five replicate samples J-flagged by the laboratory. Similar to the Time Zero tissue samples, neither DDT nor DDD were detected in any of the Day 28 tissue samples. As reported previously, the analytical results from these test sediments (Table 7) report a total DDT concentration 3.15 µg/kg (J-flagged) in sample HC-8 and 14.57 µg/kg in sample HC-10.

To determine whether there was a statistically significant difference between the reported concentrations of DDE in the Time Zero treatment and the Day 28 treatments for exposure to sediment HC-10, a paired one-tailed student's t-test was performed with a p-value of 0.05. The tissue concentrations for DDE for *Corbicula fluminea* indicate there was a significant difference between Time Zero and Time 28 day exposures in Sample HC-10.

Sample HC-08 DDE tissue concentrations were not statistically compared against the Time Zero data because there were only three samples (all J-flagged) with detectable concentrations of DDE; one replicate sample was lost during processing by the laboratory, and one sample was not detected at a MDL of 1.9 µg/kg. Hart Crowser determined that the reported results were too uncertain to conduct a valid statistical test between these two data sets.

Metals Results. The concentrations of metals in all replicate treatment samples for *Corbicula fluminea* are presented in Table 12. As previously reported, all of the reported metal concentrations in the proposed reference sediments were well below DMEF SLs. The reported tissue concentration of metals in the Day 28 samples were all similar to the Day Zero concentrations indicating no significant accumulation of metals over the 28-day exposure period. The only exception was that the mean concentration of chromium reported for exposure to test sediment HC-8 (2.6 mg/kg dry weight) was higher than the Time Zero concentration of (1.5 mg/kg dry weight). However, the higher mean concentration for the Day 28 sample is driven exclusively by one replicate sample (Replicate Sample No. 2; Lab ID K2108883-012) with a reported concentration of 9.1 mg/kg dry weight. All other replicates had a reported concentration of 1.2 mg/kg dry weight or less. It is unknown why this one replicate had such a higher concentration of chromium than the other replicates, but if this sample was removed, the mean concentration (0.925 mg/kg dry weight) is less than that reported for the Day Zero samples.

Additional Organic Compound Results. Low levels of several semivolatile compounds were detected in Corbicula fluminea tissue as presented in Table 12. These include certain PAHs, phenol and pentachlorophenol. All of these detections were J-flagged by the laboratory and are estimated values. These concentrations likely represent background levels of these compounds or are the result of matrix interference. As previously discussed, the concentrations of PAHs and other semivolatile compounds in the proposed reference sediments were either not detected at the MDL or were orders of magnitude below DMEF SLs. The one organic compound consistently detected above its MRL in all replicate samples from the three treatments was benzoic acid. However, this is a naturally occurring compound present in many living organisms and is not indicative of anthropogenic contamination.

8.6 Uncertainty Evaluation

One unexpected result from the bioaccumulation testing was the reported detections of butyltin compounds in all of the tissue sample results regardless of treatment or species. Hart Crowser requested both CAS and NAS review their

data and their laboratory practices to assist in determining potential sources for the butyltins reported in these tissue samples.

Hart Crowser also contacted TAI Environmental Services of Mobile, Alabama, to determine whether there were any potential sources of butyltins in the Cahaba River, the source of the *Corbicula fluminea* used in bioaccumulation testing. TAI Environmental Services reported the Cahaba River is not a navigable river and could be more appropriately characterized as a stream with rocky substrate amendable for *Corbicula fluminea*. No known shipyards or other industrial sources were identified in the vicinity of the collection area. There are wastewater outfalls from the City of Birmingham nearby, but the *Corbicula fluminea* were collected upstream from most of these outfalls (pers. comm. Ms. Sarah Rogers, TAI Environmental Services, January 15, 2002). It is, therefore, unlikely that the butytins found in the tissue were the result of exposure at the collection site.

CAS conducted a review of the sediment porewater and tissue data that included a review of organotin data results from other projects completed in the same time period. Their conclusion was that the butyltin results are not the result of laboratory contamination and are most likely the result of sample variation at background levels for each matrix (email from Ms. Abbie Spielman, CAS, February 5, 2002).

NAS reported they are unaware of any practices at their laboratory, including the use of cleaning and/or disinfection products, that could have introduced butyltins into either the holding or exposure tanks used in the bioaccumulation tests (pers. comm. with Dr. Richard Caldwell, Northwestern Aquatic Sciences, February 15, 2002).

Therefore, the source of the butyltins reported in the tissue and pore water samples remain uncertain. Hart Crowser recommends that if additional work is done at these reference areas, that butyltin analysis be conducted on both sediment pore water and bulk sediment to confirm the presence of butyltins. Additionally, we recommend a subset of organisms that are selected for use for bioaccumulation testing be analyzed for butyltins directly from either the laboratory culture or collection area prior to holding at the bioassay laboratory.

8.7 Bioaccumulation Testing Conclusions.

The results of this present bioaccumulation study indicate both of the tested species, the oligochaete *Lumbriculus variegates* and the bivalve *Corbicula fluminea* were acceptable as freshwater test species. There was no mortality in the test organisms over the 28-day exposure period and the organisms were

healthy at the end of the exposure period. The primary disadvantage of the oligochaete *Lumbriculus variegates* was the very limited tissue mass available for chemical analysis at the end of the test. The bivalve *Corbicula fluminea* was able to provide sufficient tissue mass at the end of the test for all of the proposed chemical analyses.

While TBT was detected in the pore water of both test sediments, neither species were found to have significantly accumulated TBT from the test sediments.

The pesticide DDT (and its metabolites DDE and DDD) were found at low levels in both proposed reference sediments. Test sediment HC-8 had a lower reported concentration of total DDT (3.15 µg/kg) than test sediment HC-10 (total DDT reported at 13.4 µg/kg). Both test species accumulated statistically greater concentrations of total DDT when exposed to sediment HC-10 versus the Time Zero sample, indicating that both these organisms can successfully be used to evaluate potential bioaccumulation of this compound. While statistical testing was only conducted on the *Lumbriculus variegates* tissue sample results comparing Time Zero to Day 28 results for exposure to test sediment HC-8, the results indicate the concentration of total DDT reported for this sediment was insufficient to cause a significant increase in tissue levels over 28-days of exposure.

9.0 STUDY CONCLUSIONS AND RECOMMENDATIONS

The following are the general conclusions and recommendations derived from this reference area study.

9.1 Study Conclusions

- This study found three reference areas that can be used as suitable reference areas for Tier III biological testing under the DMEF. All Phase II sediment samples achieved the performance standards established for 10-day bioassay testing for reference sediments under the DMEF. There is no evidence that exposure to any of these three sediments had any adverse effect on amphipod survival or midge growth and survival in these bioassays. These reference areas are: HC-2 for coarse-grained sediment located by Ross Island; HC-10 for medium-grained sediment located within Cedar Island Cove; and HC-8 for fine-grained sediment located by Elk Rock island.

- Two of these reference areas (HC-2 and HC-8) had no detectable concentrations of bioaccumulative compounds above DMEF SLs and are suitable for use as bioaccumulation study reference sediment areas.
- For the medium-grained reference location (HC-10 located within Cedar Island Cove), the pesticide DDT (and its metabolites) were not detected during the Phase I reconnaissance portion of this study but were detected at levels exceeding DMEF SLs during the Phase II portion of the study. This location's use as a bioaccumulation testing reference site when DDT is a contaminant of concern should be further evaluated by additional chemical testing of Cedar Island Cove to identify the extent of DDT present at this location.
- The Phase I Reconnaissance portion of this study found nine locations within the LWR of varying grain size with no detected concentrations of pesticides or PCBs in surface sediment and limited detection of petroleum hydrocarbons. Three of these areas were evaluated in Phase II. The remaining six areas could be further evaluated for use as reference areas.
- The results of the bioaccumulation study indicate both of the tested species, the oligochaete *Lumbriculus variegates* and the bivalve *Corbicula fluminea*, were acceptable as freshwater test species based on survival during the 28-day test period and similar accumulation characteristics for the bioaccumulative compounds found in the test sediments.
- The bioaccumulation study results confirmed that *Corbicula fluminea* is preferable over *Lumbriculus variegates* because its greater tissue mass allows for more flexibility in conducting chemical analysis of tissue.

9.2 Study Recommendations

- Supplemental sampling conducted at Cedar Island Cove indicated there is a range of sediment grain sizes available at this location, and it is likely this is also true at Elk Rock Island. Additional sediment grain size analysis at Elk Rock Island can be used to confirm this assumption.
- Additional chemical monitoring of Elk Rock Island and Cedar Island Cove is recommended to assess their chemical stability over time. This monitoring could also be used to identify potential sources of DDT (and its metabolites) detected under the Phase II portion of this study.
- Additional work should be pursued to identify background levels of butyltins in bulk sediment and pore water. Additionally, if additional sediment analysis for butyltins is conducted, it is recommended that both pore water and bulk sediment analysis be performed.

- It is recommended that a subset of organisms that are selected for use for bioaccumulation testing be analyzed for butyltins directly from either the laboratory culture or collection area prior to holding at the bioassay laboratory. This should help reduce the uncertainty associated with the ubiquitous butytin detections found in this present study.

10.0 REFERENCES

ASTM, 1995. Standard Test Methods for Measuring the Toxicity of Sediment-Associated Contaminants with Fresh Water Invertebrates. E 1706-95.

Corps, 1996. Report of Dredge Test Analysis; W.O. #97-077. Lower Willamette River Portland Harbor Project. December 6, 1996.

Corps et al., 1998. Dredged Material Evaluation Framework: Lower Columbia River Management Area. Prepared by U.S. Army Corps of Engineers, U.S. Environmental Protection Agency, Oregon Department of Environmental Quality, Washington Department of Ecology, and Washington Department of Natural Resources. November 1998.

Ecology, 1995. Guidance on the Development of Sediment Sampling and Analysis Plans Meeting the Requirements of the Sediment Management Standards (Chapter 173-204 WAC). Washington Department of Ecology. December 1995.

EPA/Corps, 1998. Evaluation of Dredged Material Proposed for Discharge in Waters of the U.S – Inland Testing Manual. March 1998.

EPA, 2000. Methods for Measuring the Toxicity and Bioaccumulation of Sediment-Associated Contaminants with Freshwater Invertebrates. Second Edition. Office of Water EPA/600/R-99/064.

Hart Crowser, 1997a. Sediment Characterization Study; River Terminals 1, 2, and 5. Prepared for the Port of Portland. January 14, 1997.

Hart Crowser, 1997b. Sediment Characterization Study; River Terminal 4, Slip 3; Willamette River; Portland, Oregon. Prepared for the Port of Portland. August 29, 1997.

Hart Crowser, 1998. Sediment Characterization Study; Terminal 4, Berth 416; Willamette River, Portland, Oregon. Prepared for the Port of Portland. March 10, 1998.

Hart Crowser, 1999a. Sediment Characterization Study; Marine Terminal 2, Berths 203-206; Portland, Oregon. Prepared for the Port of Portland. February 2, 1999.

Hart Crowser, 1999b. Sediment Characterization Study; Marine Terminal 4, Berth 416; Willamette River, Portland, Oregon. Prepared for the Port of Portland. April 20, 1999.

Hart Crowser, 2000a. Remedial Investigation Report; Terminal 4, Slip 3; Port of Portland, Portland, Oregon. Prepared for the Port of Portland. April 18, 2000.

Hart Crowser, 2000b. Site Investigation Report, Port of Portland, Confined Dredged Material Disposal, Ross Island Facility, Portland, Oregon. Prepared for the Port of Portland. November 30, 2000.

Hart Crowser, 2001c. Sampling and Analysis Plan, Willamette River Reference Area Study, U.S. Army Corps of Engineers, Portland, Oregon. August 27, 2001.

Hart Crowser, 2001d. Willamette River Reference Area Study – Phase I Results and Recommendations for Phase II Sampling Locations. September 27, 2001.

Hill, S., and P. McLaren, 2001. A Sediment Trend Analysis of the Lower Willamette River. August 2001.

Table 1 - Phase I Sampling Locations and Elevations
Lower Willamette River Reference Area Study
Portland, Oregon

Sample Identification	Northing Coordinate	Easting Coordinate	Approximate Mudline Elevation (in CRD)
HC-1	667283.0	7648081.0	-5.3
HC-2	668169.0	7646915.0	-16.3
HC-3	662050.5	7646279.0	-10.3
HC-4	660312.0	7647220.0	-24
HC-5	656954.0	7648651.0	-25.5
HC-6	653165.0	7651568.0	No Sample
HC-7	653151.5	7650650.9	-29.5
HC-8	652813.1	7651280.8	-5.8
HC-9	635733.8	7655487.5	-6.5
HC-10	634435.3	7655924.6	-10.8

F:\DATA\Jobs\7402-01 Corps Reference Site\Reports 7402-01\Final Report\Tables 1-10

Notes:
1. State Plane Coordinates in NAD 83 Oregon North.
2. Mudline Elevation = water depth in feet. Columbia River Datum (CRD) corrected for tide and river stage.
3. No sample was collected at location HC-6 as no acceptable sediment recovery was achieved.

Table 2 - Phase I Sediment Physical Characteristics
Lower Willamette River Reference Area Study
Portland, Oregon

Sample Identification	Field Wet Sieve Results % Silt/Clay	Laboratory Sieve Analysis Results % Sand/Gravel > 75 µm	Laboratory Sieve Analysis Results % Silt/Clay <75 µm	Material Description
HC-1	9.0	95.0	5.0	Slightly silty, medium to fine SAND
HC-2	16.0	90.2	9.8	Slightly silty, medium to fine SAND
HC-3	54.0	39.1	60.9	Very sandy SILT
HC-4	62.0	30.7	69.3	Very sandy SILT
HC-5	23.0	74.9	25.1	Silty, fine SAND
HC-7	6.0	96.0	4.0	Slightly gravelly SAND
HC-8	53.0	35.1	64.9	Very sandy SILT
HC-9	46.0	56.6	43.4	Very silty, fine SAND
HC-10	72.0	40.3	59.7	Very sandy SILT

F:\DATA\Jobs\7402-01 Corps Reference Site\Reports 7402-01\Final Report\Tables 1-10

Note:

% Silt/Clay = % Fines (e.g., material less than 75 µm).

Table 3 - Analytical Results for Phase I Sediment Samples
Lower Willamette River Reference Area Study
Portland, Oregon

Sample ID	HC-1	HC-2	HC-3	HC-4	HC-5	HC-7	HC-8	HC-9	HC-10
Conventionals in percent									
Total Volatile Solids in %	3.35	3.97	6.03	7.11	4.27	2.35	7.23	6.5	6.65
Total Solids in %	73.4	70.7	57.7	52.1	66.9	77.9	54.1	59.3	50.9
TPH in mg/kg									
Gasoline Range Organics	14 U	14 U	33 U	37 U	28 U	13 U	35 U	28 U	34 U
Diesel Range Organics	14 U	14 U	34 Z	56 Z	28 U	13 U	39 Z	28 U	38 Z
Residual Range Organics	34 U	35 U	190 Z	290 Z	72 Z	32 U	230 Z	160 Z	200 Z
Pesticides in ug/kg									
Alpha-BHC	1.4 U	1.5 U	1.8 U	2.0 U	1.5 U	1.3 U	1.9 U	1.7 U	2.0 U
Beta-BHC	1.4 U	1.5 U	1.8 U	2.0 U	1.5 U	1.3 U	1.9 U	1.7 U	2.0 U
Gamma-BHC (Lindane)	1.4 U	1.5 U	1.8 U	2.0 U	1.5 U	1.3 U	1.9 U	1.7 U	2.0 U
Delta-BHC	1.4 U	1.5 U	1.8 U	2.0 U	1.5 U	1.3 U	1.9 U	1.7 U	2.0 U
Heptachlor	1.4 U	1.5 U	1.8 U	2.0 U	1.5 U	1.3 U	1.9 U	1.7 U	2.0 U
Aldrin	1.4 U	1.5 U	1.8 U	2.0 U	1.5 U	1.3 U	1.9 U	1.7 U	2.0 U
Heptachlor Epoxide	1.4 U	1.5 U	1.8 U	2.0 U	1.5 U	1.3 U	1.9 U	1.7 U	2.0 U
Gamma-Chlordane	1.4 U	1.5 U	1.8 U	2.0 U	1.5 U	1.3 U	1.9 U	1.7 U	2.0 U
Endosulfan I	1.4 U	1.5 U	1.8 U	2.0 U	1.5 U	1.3 U	1.9 U	1.7 U	2.0 U
Alpha-Chlordane	1.4 U	1.5 U	1.8 U	2.0 U	1.5 U	1.3 U	1.9 U	1.7 U	2.0 U
Dieldrin	1.4 U	1.5 U	1.8 U	2.0 U	1.5 U	1.3 U	1.9 U	1.7 U	2.0 U
4,4'-DDE	1.4 U	1.5 U	1.8 U	2.0 U	1.5 U	1.3 U	1.9 U	1.7 U	2.0 U
Endrin	1.4 U	1.5 U	1.8 U	2.0 U	1.5 U	1.3 U	1.9 U	1.7 U	2.0 U
Endosulfan II	1.4 U	1.5 U	1.8 U	2.0 U	1.5 U	1.3 U	1.9 U	1.7 U	2.0 U
4,4'-DDD	1.4 U	1.5 U	1.8 U	2.0 U	1.5 U	1.3 U	1.9 U	1.7 U	2.0 U
Endrin Aldehyde	1.4 U	1.5 U	1.8 U	2.0 U	1.5 U	1.3 U	1.9 U	1.7 U	2.0 U
Endosulfan Sulfate	1.4 U	1.5 U	1.8 U	2.0 U	1.5 U	1.3 U	1.9 U	1.7 U	2.0 U
4,4'-DDT	1.4 U	1.5 U	1.8 U	2.0 U	1.5 U	1.3 U	1.9 U	1.7 U	2.0 U
Endrin Ketone	1.4 U	1.5 U	1.8 U	2.0 U	1.5 U	1.3 U	1.9 U	1.7 U	2.0 U
Methoxychlor	1.4 U	1.5 U	1.8 U	2.0 U	1.5 U	1.3 U	1.9 U	1.7 U	2.0 U
Toxaphene	6.9 U	71 U	87 U	96 U	75 U	65 U	93 U	85 U	98 U
PCBs in ug/kg									
Aroclor 1016	14 U	15 U	18 U	20 U	15 U	13 U	19 U	17 U	20 U
Aroclor 1221	28 U	29 U	35 U	39 U	30 U	26 U	37 U	34 U	40 U
Aroclor 1232	14 U	15 U	18 U	20 U	15 U	13 U	19 U	17 U	20 U
Aroclor 1242	14 U	15 U	18 U	20 U	15 U	13 U	19 U	17 U	20 U
Aroclor 1248	14 U	15 U	18 U	20 U	15 U	13 U	19 U	17 U	20 U
Aroclor 1254	14 U	15 U	18 U	20 U	15 U	13 U	19 U	17 U	20 U
Aroclor 1260	14 U	15 U	18 U	20 U	15 U	13 U	19 U	17 U	20 U
Total PCBs	28 U	29 U	35 U	39 U	30 U	26 U	37 U	34 U	40 U

Notes:

U = Not detected at the indicated method reporting limits (MRL).

Z = The chromatographic fingerprint does not resemble a petroleum product (most likley a non-polar biogenic oil).

Table 4 - Decision Matrix for Phase II Sample Selection
Lower Willamette River Reference Area Study
Portland, Oregon

1. Fine-Grained Phase I Samples

Decision Criteria	Sample ID		
	HC-3	HC-4	HC-8
Analytical Chemistry	5	5	5
Stability of Location	2	2	5
Grain Size Match	3	4	3
Total Score	10	11	13

2. Medium-Grained Phase I Samples

Decision Criteria	Sample ID		
	HC-5	HC-9	HC-10
Analytical Chemistry	5	5	5
Stability of Location	2	4	5
Grain Size Match	2	4	5
Total Score	9	13	15

3. Coarse-Grained Phase I Samples

Decision Criteria	Sample ID		
	HC-1	HC-2	HC-7
Analytical Chemistry	5	5	5
Stability of Location	2	2	2
Grain Size Match	5	5	5
Total Score	12	12	12

F:\DATA\Jobs\7402-01 Corps Reference Site\Reports 7402-01\Final Report\Tables 1-10

Note:
Scores for each category presented in the decision matrix are subjective scores based on a scale from 1 to 5, with 5 indicating the highest match with program objectives.

Table 5 - Phase II Sampling Locations and Elevations
Lower Willamette River Reference Area Study
Portland, Oregon

Sample Identification	Northing Coordinate[1]	Easting Coordinate[1]	Approximate Mudline Elevation (in CRD)[2]
HC-2	668155	7646929	-5.9
HC-8	652810	7651279	-6.5
HC-10	634420	7655918	-11.8
CD-1	634174	7656037	--
CD-2	634656	7655728	--
CD-3	635089	7655561	--
CD-4	635700	7655435	--
CD-5	636020	7655355	--

F:\DATA\Jobs\7402-01 Corps Reference Site\Reports 7402-01\Final Report\Tables 1-10

Notes:

(1) State Plane Coordinates in NAD 83 Oregon North.

(2) All Elevations presented are in feet CRD.

-- = Not Measured.

Samples CD-1 through CD-5 were supplemental samples collected to assess the range of sediment grain sizes present in Cedar Island Cove (Figure 5).

Table 6 - Phase II Sediment Physical Characteristics
Lower Willamette River Reference Area Study
Portland, Oregon

Sample Identification	% Sand/Gravel >63 µm	% Silt/Clay[1] <63 µm	TVS[2] Percent	TOC[3] Percent	Material Description
HC-2	93.9	6.1	2.6	0.4	Slightly silty, medium to fine SAND
HC-8	33.7	66.3	6.2	1.8	Clayey, sandy SILT
HC-10	47.2	52.8	5.0	1.3	Clayey, very sandy SILT
CD-1	10.1	89.9	7.4	2.3	Slightly sandy, very clayey SILT
CD-2	48.9	51.1	4.9	1.2	Clayey, very sandy SILT
CD-3	26.8	73.2	6.4	2.0	Clayey, sandy SILT
CD-4	78.6	21.4	4.4	1.2	Slightly clayey, silty, medium to fine SAND
CD-5	61.5	38.5	4.8	1.5	Slightly clayey, very silty, medium to fine SAND

F:\DATA\Jobs\7402-01 Corps Reference Site\Reports 7402-01\Final Report\Tables 1-10

Notes:
(1) % Silt/Clay = % Fines (e.g., material less than 63 µm).
(2) TVS = Total Volatile Solids.
(3) TOC = Total Organic Carbon.

Table 7 - Analytical Results of Phase II Sediment Samples
Lower Willamette River Reference Area Study
Portland, Oregon

Sample-ID		HC-2	HC-8	HC-10
Lab-ID	DMEF	K2106748-001	K2106748-002	K2106748-003
Sediment Type		Coarse-grained	Fine-grained	Medium-grained
Sampling Date	SL	9/17/2001	9/17/2001	9/17/2001
Conventionals in percent				
Carbon, Total Organic (TOC)		0.42	1.77	1.3
Solids, Total Volatile		2.62	6.16	5
Metals in mg/kg				
Antimony, Total	150	0.47 J	0.06 J	0.05 J
Arsenic, Total	57	2.7	4.3	3.3
Cadmium, Total	5.1	0.07	0.18	0.11
Chromium, Total		16.8	40.5	29.4
Copper, Total	390	15.4	50.9	34.1
Lead, Total	450	5.24	12.6	9.21
Mercury, Total	0.41	0.02	0.05	0.04
Nickel, Total	140	18.5	35.7	27.2
Silver, Total	6.1	0.1	0.22	0.15
Zinc, Total	410	49.7	96.8	72.7
Organometallics in µg/L				
Tetra-n-butyltin		0.072 U	0.05 U	0.05 U
Tri-n-butyltin (TBT)	0.15	0.07	0.07	0.064
Di-n-butyltin		0.037 J	0.035 J	0.017 J
n-Butyltin		0.072 U	0.05 U	0.05 U
LPAHs in µg/kg				
Acenaphthene	500	16 U	21 U	19 U
Acenaphthylene	560	3.2 J	4.5 J	3.5 J
Anthracene	960	16 U	21 U	5 J
Fluorene	540	16 U	21 U	19 U
Naphthalene	2100	3.3 J	7.8 J	6.1 J
Phenanthrene	1500	4.3 J	11 J	14 J
Total LPAHs	5200	10.8 J	23.3 J	28.6 J
HPAHs in µg/kg				
Benz(a)anthracene	1300	4.8 J	8.1 J	13 J
Benzo(a)pyrene	1600	13 J	8.8 J	13 J
Benzo(b)fluoranthene		6.6 J	11 J	14 J
Benzo(g,h,i)perylene	670	45	9.6 J	14 J
Benzo(k)fluoranthene		5 J	4.4 J	5.2 J
Chrysene	1400	5.1 J	9.5 J	13 J
Dibenz(a,h)anthracene	230	16 U	21 U	19 U
Fluoranthene	1700	5.6 J	18 J	21
Indeno(1,2,3-cd)pyrene	600	12 J	8.2 J	8 J
Pyrene	2600	10 J	19 J	30
Total Benzofluoranthenes	3200	11.7 J	15.4 J	19.2 J
Total HPAHs	12000	107.1	96.6 J	131.2
Phenols in µg/kg				
2,4-Dimethylphenol	29	76 U	110 U	94 U
2-Methylphenol	63	16 U	21 U	19 U
4-Methylphenol	670	16 U	11 J	7.3 J
Pentachlorophenol (PCP)	400	150 U	210 U	190 U
Phenol	420	46 U	63 U	11 J
Phthalates in µg/kg				
Bis(2-ethylhexyl) Phthalate	8300	310 U	420 U	380 U
Butyl Benzyl Phthalate	970	16 U	21 U	19 U
Di-n-butyl Phthalate	5100	16 U	9 J	6.5 J

Please refer to notes at end of table.

Table 7 - Analytical Results of Phase II Sediment Samples
Lower Willamette River Reference Area Study
Portland, Oregon

Sample-ID		HC-2	HC-8	HC-10
Lab-ID	DMEF	K2106748-001	K2106748-002	K2106748-003
Sediment Type		Coarse-grained	Fine-grained	Medium-grained
Sampling Date	SL	9/17/2001	9/17/2001	9/17/2001
Di-n-octyl Phthalate	6200	16 U	21 U	19 U
Diethyl Phthalate	1200	16 U	21 U	19 U
Dimethyl Phthalate	1400	16 U	21 U	19 U
Semivolatiles in µg/kg				
Benzoic Acid	650	310 U	64 J	35 J
Benzyl Alcohol	57	16 U	21 U	19 U
Dibenzofuran	540	16 U	21 U	19 U
Hexachlorobenzene	22	16 U	21 U	19 U
Hexachlorobutadiene	29	16 U	21 U	19 U
N-Nitrosodiphenylamine	28	16 U	21 U	19 U
Volatiles in µg/kg				
1,2-Dichlorobenzene	35	16 U	21 U	19 U
1,3-Dichlorobenzene	170	16 U	21 U	19 U
1,4-Dichlorobenzene	110	16 U	21 U	19 U
Pesticide/PCBs in µg/kg				
4,4'-DDD		1.6 U	0.99 JP	0.77 J
4,4'-DDE		1.6 U	0.76 J	0.8 J
4,4'-DDT		0.73 JP	1.4 JP	13
Total DDT	6.9	0.73 JP	3.15 J	14.57
Aldrin	10	1.6 U	2.1 U	1.9 U
Aroclor 1016		16 U	21 U	19 U
Aroclor 1221		31 U	42 U	38 U
Aroclor 1232		16 U	21 U	19 U
Aroclor 1242		16 U	21 U	19 U
Aroclor 1248		16 U	21 U	19 U
Aroclor 1254		16 U	21 U	19 U
Aroclor 1260		16 U	21 U	19 U
Total PCBs	130	16 U	21 U	19 U
Chlordane	10			
Dieldrin	10	1.6 U	2.1 U	1.9 U
Endosulfan I		1.6 U	2.1 U	1.9 U
Endosulfan II		1.6 U	2.1 U	1.9 U
Endosulfan Sulfate		1.6 U	2.1 U	1.9 U
Endrin		1.6 U	2.1 U	1.9 U
Endrin Aldehyde		1.6 U	2.1 U	1.9 U
Endrin Ketone		1.6 U	2.1 U	1.9 U
Heptachlor	10	1.6 U	2.1 U	1.9 U
Heptachlor Epoxide		1.6 U	2.1 U	1.9 U
Methoxychlor		1.6 U	2.1 U	1.9 U
Toxaphene		76 U	110 U	93 U
alpha-BHC		1.6 U	2.1 U	1.9 U
alpha-Chlordane		1.6 U	2.1 U	1.9 U
beta-BHC		1.6 U	2.1 U	1.9 U
delta-BHC		1.6 U	2.1 U	1.9 U
gamma-BHC (Lindane)	10	1.6 U	2.1 U	1.9 U
gamma-Chlordane		1.6 U	2.1 U	1.9 U

F:\DATA\Jobs\7402-01 Corps Reference Site\Reports 7402-01\Final Report\Tables 1-10

Notes:

Exceeds DMEF SL.

U = Not detected at the indicated method reporting limits (MRL).

J = Estimated concentration that is less than the MRL but greater than or equal to the MDL.

P = The GC or HPLC confirmation criteria were exceeded.

Table 8 - Summary of Phase II Sediment Bioassay Results
Amphipod Survival Bioassay (*Hyalella azteca*)
Lower Willamette River Reference Area Study
Portland, Oregon

Sample Identification	Test Mean Mortality in percent	Performance Criteria[1]	Performance Criteria Met?
HC-2	7.5	< 30% Mortality	Yes
HC-8	6.3	< 30% Mortality	Yes
HC-10	6.3	< 30% Mortality	Yes
Neg. Control	6.3	< 20% Mortality	Yes

F:\DATA\Jobs\7402-01 Corps Reference Site\Reports 7402-01\Final Report\Tables 1-10

Note:
(1) Corps et al., 1998 DMEF.

Table 9 - Summary of Phase II Sediment Bioassay Results
Midge Survival Bioassay (*Chironomus tentans*)
Lower Willamette River Reference Area Study
Portland, Oregon

Sample Identification	Test Mean Mortality in percent	Performance Criteria[1]	Performance Criteria Met?
HC-2	13.8	< 35% Mortality	Yes
HC-8	7.5	< 35% Mortality	Yes
HC-10	16.3	< 35% Mortality	Yes
Neg. Control	18.8	< 30% Mortality	Yes

F:\DATA\Jobs\7402-01 Corps Reference Site\Reports 7402-01\Final Report\Tables 1-10

Note:
(1) Corps et al., 1998 DMEF.

Table 10 - Summary of Phase II Sediment Bioassay Results
Midge Growth Bioassay (*Chironomus tentans*)
Lower Willamette River Reference Area Study
Portland, Oregon

Sample Identification	Test Mean Individual Biomass in mg	Performance Criteria[1]	Performance Criteria Met?
HC-2	1.03	0.6 mg minimum mean individual biomass	Yes
HC-8	0.92	0.6 mg minimum mean individual biomass	Yes
HC-10	1.07	0.6 mg minimum mean individual biomass	Yes
Neg. Control	0.88	0.6 mg minimum mean individual biomass	Yes

F:\DATA\Jobs\7402-01 Corps Reference Site\Reports 7402-01\Final Report\Tables 1-10

Note:
(1) Corps et al., 1998 DMEF.

Table 11 - Tissue Results from Bioaccumulation Testing for *Lumbriculus variegatus*
Lower Willamette River Reference Area Study
Portland, Oregon

Sample ID	Day 0						Day 28 for Sediment Sample HC-08 Exposure					HC-08	Day 28 for Sediment Sample HC-10 Exposure					HC-10
Replicate Number	1	2	3	4	5	Day 0	1	2	3	4	5	Day 28	1	2	3	4	5	Day 28
Lab ID	K2107717-001	K2107717-002	K2107717-003	K2107717-004	K2107717-005	Mean	K21108269-006	K21108269-007	K21108269-008	K21108269-009	K21108269-010	Mean	K21108269-011	K21108269-012	K21108269-013	K21108269-014	K21108269-015	Mean
Conventionals as Wet Wt.																		
Sample Wt. in grams	25.36	23.77	23.99	22.98	24.05	**24.03**	9.46	7.05	7.92	8.17	9.85	**8.49**	9.42	7.31	8.69	8.39	8.67	**8.50**
Total Lipids in %	1.22	1.03	1.46	1.12	1.02	**1.17**	1.01	1.11	1.12	0.99	0.88	**1.02**	0.95	1.03	0.97	0.97	0.96	**0.98**
Total Solids in %	14.9	13.8	13.9	13.5	12.8	**13.8**	na	na	na	na	na	--	na	na	na	na	na	--
Metals in mg/kg as Dry Wt																		
Antimony, Total	0.02 U	0.02 U	0.02 U	0.02 U	0.02 U	--	na	na	na	na	na	--	na	na	na	na	na	--
Arsenic, Total	**6.9**	**7.2**	**6.4**	**7.4**	**7.1**	**7**	na	na	na	na	na	--	na	na	na	na	na	--
Cadmium, Total	**0.15**	**0.15**	**0.13**	**0.16**	**0.15**	**0.15**	na	na	na	na	na	--	na	na	na	na	na	--
Chromium, Total	0.4 U	0.4 U	0.4 U	0.4 U	0.4 U	--	na	na	na	na	na	--	na	na	na	na	na	--
Copper, Total	11.1 U	11.6 U	10.9 U	12.7 U	**13.5** J	**7.3**	na	na	na	na	na	--	na	na	na	na	na	--
Lead, Total	**0.63**	**0.75**	**0.6**	**0.65**	**0.73**	**0.67**	na	na	na	na	na	--	na	na	na	na	na	--
Mercury, Total	**0.44**	**0.44**	**0.44**	**0.46**	**0.43**	**0.44**	na	na	na	na	na	--	na	na	na	na	na	--
Nickel, Total	**0.31**	**0.34**	**0.33**	**0.35**	**0.36**	**0.34**	na	na	na	na	na	--	na	na	na	na	na	--
Silver, Total	**0.06**	**0.06**	**0.06**	**0.07**	**0.07**	**0.06**	na	na	na	na	na	--	na	na	na	na	na	--
Zinc, Total	**353**	**353**	**313**	**364**	**354**	**347.4**	na	na	na	na	na	--	na	na	na	na	na	--
Butyltins in µg/kg as Wet Wt.																		
Tetra-n-butyltin	1.1 U	1.2 U	1.4 U	1.4 U	1.2 U	--	3.7 U	10 U	10 U	10 U	3.3 U	--	3.5 U	10 U	5.7 U	5.4 U	5 U	--
Tri-n-butyltin (TBT)	1.1 U	1.2 U	1.4 U	1.4 U	1.2 U	--	3.7 U	10 U	10 U	10 U	3.3 U	--	3.5 U	10 U	5.7 U	5.4 U	5 U	--
Di-n-butyltin	**12**	**18** J	**15**	**11**	**12**	**13.6**	**1.4** J	**2.2** J	**2.4** J	**3.3** J	**3.9**	**2.6**	**2.6** J	**3.2** J	**3.7** J	**2.7** J	**3.9** J	**3.2**
n-Butyltin	**7.1**	**10** J	**7.4**	**4.1**	**5.4**	**6.8**	**6.2**	**11**	**11**	**8.3** J	**14**	**10.1**	**11**	**9.3** J	**7.5**	**8.8**	**7**	**8.7**
Semivolatiles in µg/kg as Wet Wt.																		
Acenaphthene	80 U	80 U	80 U	80 U	80 U	--	na	na	na	na	na	--	na	na	na	na	na	--
Acenaphthylene	80 U	80 U	80 U	80 U	80 U	--	na	na	na	na	na	--	na	na	na	na	na	--
Anthracene	80 U	80 U	80 U	80 U	80 U	--	na	na	na	na	na	--	na	na	na	na	na	--
Fluorene	80 U	80 U	80 U	80 U	80 U	--	na	na	na	na	na	--	na	na	na	na	na	--
Naphthalene	80 U	80 U	80 U	80 U	80 U	--	na	na	na	na	na	--	na	na	na	na	na	--
Phenanthrene	80 U	80 U	80 U	80 U	80 U	--	na	na	na	na	na	--	na	na	na	na	na	--
2-Methylnaphthalene	80 U	80 U	80 U	80 U	80 U	--	na	na	na	na	na	--	na	na	na	na	na	--
Benz(a)anthracene	80 U	80 U	80 U	80 U	80 U	--	na	na	na	na	na	--	na	na	na	na	na	--
Benzo(a)pyrene	80 U	80 U	80 U	80 U	80 U	--	na	na	na	na	na	--	na	na	na	na	na	--
Benzo(b)fluoranthene	80 U	80 U	80 U	80 U	80 U	--	na	na	na	na	na	--	na	na	na	na	na	--
Benzo(k)fluoranthene	80 U	80 U	80 U	80 U	80 U	--	na	na	na	na	na	--	na	na	na	na	na	--
Benzo(g,h,i)perylene	**19** J	80 U	80 U	80 U	80 U		na	na	na	na	na	--	na	na	na	na	na	--
Chrysene	80 U	80 U	80 U	80 U	80 U	--	na	na	na	na	na	--	na	na	na	na	na	--
Dibenz(a,h)anthracene	80 U	80 U	80 U	80 U	80 U	--	na	na	na	na	na	--	na	na	na	na	na	--
Fluoranthene	80 U	80 U	80 U	80 U	80 U	--	na	na	na	na	na	--	na	na	na	na	na	--
Indeno(1,2,3-cd)pyrene	80 U	80 U	80 U	80 U	80 U	--	na	na	na	na	na	--	na	na	na	na	na	--
Pyrene	80 U	80 U	80 U	80 U	80 U	--	na	na	na	na	na	--	na	na	na	na	na	--
Phenols in µg/kg as Wet Wt.																		
2,4-Dimethylphenol	160 U	**48** J	160 U	**34** J	**160** J	**52**	na	na	na	na	na	--	na	na	na	na	na	--
2-Methylphenol	160 U	160 U	160 U	**68** J	**70** J	**46.8**	na	na	na	na	na	--	na	na	na	na	na	--
4-Methylphenol	**1300** J	**1100**	**1100**	**1500** J	**1500**	**1300**	na	na	na	na	na	--	na	na	na	na	na	--
Pentachlorophenol (PCP)	**630** J	640 U	640 U	640 U	640 U	**262**	na	na	na	na	na	--	na	na	na	na	na	--
Phenol	**720** J	**610**	**590**	**700** J	**690**	**662**	na	na	na	na	na	--	na	na	na	na	na	--
Misc. Semivolatiles in µg/kg as Wet Wt.																		
Benzoic Acid	3200 U	3200 U	3200 U	3200 U	3200 U	--	na	na	na	na	na	--	na	na	na	na	na	--
Benzyl Alcohol	**1500**	**650**	**610**	**1100**	**910**	**954**	na	na	na	na	na	--	na	na	na	na	na	--
Dibenzofuran	80 U	80 U	80 U	80 U	80 U	--	na	na	na	na	na	--	na	na	na	na	na	--
Hexachlorobutadiene	80 U	80 U	80 U	80 U	80 U	--	na	na	na	na	na	--	na	na	na	na	na	--
Hexachloroethane	80 U	80 U	80 U	80 U	80 U	--	na	na	na	na	na	--	na	na	na	na	na	--
N-Nitrosodiphenylamine	160 U	160 U	160 U	160 U	160 U	--	na	na	na	na	na	--	na	na	na	na	na	--
1,2-Dichlorobenzene	80 U	80 U	80 U	80 U	80 U	--	na	na	na	na	na	--	na	na	na	na	na	--
1,3-Dichlorobenzene	80 U	80 U	80 U	80 U	80 U	--	na	na	na	na	na	--	na	na	na	na	na	--
1,4-Dichlorobenzene	80 U	80 U	80 U	80 U	80 U	--	na	na	na	na	na	--	na	na	na	na	na	--
1,2,4-Trichlorobenzene	80 U	80 U	80 U	80 U	80 U	--	na	na	na	na	na	--	na	na	na	na	na	--
Hexachlorobenzene	80 U	80 U	80 U	80 U	80 U	--	na	na	na	na	na	--	na	na	na	na	na	--

Please refer to notes at the end of this table.

Table 11 - Tissue Results from Bioaccumulation Testing for *Lumbriculus variegatus*
Lower Willamette River Reference Area Study
Portland, Oregon

Sample ID	Day 0						Day 28 for Sediment Sample HC-08 Exposure					HC-08	Day 28 for Sediment Sample HC-10 Exposure					HC-10
Replicate Number	1	2	3	4	5	Day 0	1	2	3	4	5	Day 28	1	2	3	4	5	Day 28
Lab ID	K2107717-001	K2107717-002	K2107717-003	K2107717-004	K2107717-005	Mean	K21108269-006	K21108269-007	K21108269-008	K21108269-009	K21108269-010	Mean	K21108269-011	K21108269-012	K21108269-013	K21108269-014	K21108269-015	Mean
Phthalates in µg/kg																		
Bis(2-ethylhexyl) Phthalate	**180**	160 U	160 U	160 U	160 U	**150**	na	na	na	na	na	--	na	na	na	na	na	--
Butyl Benzyl Phthalate	80 U	80 U	80 U	80 U	80 U	--	na	na	na	na	na	--	na	na	na	na	na	--
Di-n-butyl Phthalate	80 U	80 U	80 U	80 U	80 U	--	na	na	na	na	na	--	na	na	na	na	na	--
Di-n-octyl Phthalate	80 U	80 U	80 U	80 U	80 U	--	na	na	na	na	na	--	na	na	na	na	na	--
Diethyl Phthalate	80 U	80 U	80 U	80 U	80 U	--	na	na	na	na	na	--	na	na	na	na	na	--
Dimethyl Phthalate	80 U	80 U	80 U	80 U	80 U	--	na	na	na	na	na	--	na	na	na	na	na	--
Pesticides/PCBs in µg/kg as Wet Wt.																		
4,4'-DDD	2 U	**0.7** J	2 U	**0.83** J	**1.6** JP	**0.75**	2 U	2 U	2 U	2 U	2 U	--	**0.94** J	**1.1** J	**0.97** J	**0.98** J	**0.94** J	**0.9**
4,4'-DDE	**1.5** JP	**1.5** JP	**1.6** JP	**1.6** JP	**2.1** P	**1.66**	**1.9** J	**1.9** J	**1.9** J	**2** J	**2.1** J	**2.0**	**3.7** J	**4.6** J	**6.1** J	**3.7** J	**3.6** J	**4.3**
4,4'-DDT	**0.76** J	**0.86** J	**0.83** J	2 U	**1** JP	**0.73**	2 U	2 U	2 U	2 U	2 U	--	2 U	2 U	4 U	2 U	2 U	--
Total DDT	**2.26** JP	**3.06** JP	**2.43** JP	**2.43** JP	**4.7** JP	**3.14**	**1.9** J	**1.9** J	**1.9** J	**2** J	**2.1** J	**2.0**	**4.64** J	**5.7** J	**7.07** J	**4.7** J	**4.5** J	**4.8**
Aldrin	2 U	2 U	2 U	2 U	2 U	--	2 U	2 U	2 U	2 U	2 U	--	2 U	2 U	4 U	2 U	2 U	--
alpha-Chlordane	2.5 U	2.4 U	2 U	2.5 U	2.8 U	--	2 U	2 U	2 U	6.4 U	7.6 U	--	2 U	5.7 U	4 U	2 U	2 U	--
gamma-Chlordane	2 U	2 U	2 U	2 U	2 U	--	2 U	2 U	2 U	2 U	2 U	--	2 U	2 U	4 U	2 U	2 U	--
Dieldrin	2 U	2 U	2 U	2 U	2 U	--	2 U	2 U	2 U	2 U	2 U	--	2 U	2 U	4 U	2 U	2 U	--
Heptachlor	2 U	2 U	2 U	2 U	**0.8** JP	**0.29**	2 U	2 U	2 U	2 U	2 U	--	2 U	2 U	4 U	2 U	2 U	--
gamma-BHC (Lindane)	2 U	2 U	2 U	2 U	2 U	--	2 U	2 U	2 U	2 U	2 U	--	2 U	2 U	4 U	2 U	2 U	--
Aroclor 1016	20 U	20 U	20 U	20 U	20 U	--	1.7 U	1.7 U	1.7 U	1.7 U	1.7 U	--	1.7 U	1.7 U	1.7 U	1.7 U	1.7 U	--
Aroclor 1221	40 U	40 U	40 U	40 U	40 U	--	3.4 U	3.4 U	3.4 U	3.4 U	3.4 U	--	3.4 U	3.4 U	3.4 U	3.4 U	3.4 U	--
Aroclor 1232	20 U	20 U	20 U	20 U	20 U	--	1.7 U	1.7 U	1.7 U	1.7 U	1.7 U	--	1.7 U	1.7 U	1.7 U	1.7 U	1.7 U	--
Aroclor 1242	20 U	20 U	20 U	20 U	20 U	--	1.7 U	1.7 U	1.7 U	1.7 U	1.7 U	--	1.7 U	1.7 U	1.7 U	1.7 U	1.7 U	--
Aroclor 1248	20 U	20 U	20 U	20 U	20 U	--	1.7 U	1.7 U	1.7 U	1.7 U	1.7 U	--	1.7 U	1.7 U	1.7 U	1.7 U	1.7 U	--
Aroclor 1254	20 U	20 U	20 U	**15** J	**12** J	**8.4**	1.7 U	1.7 U	1.7 U	1.7 U	1.7 U	--	1.7 U	1.7 U	1.7 U	1.7 U	1.7 U	--
Aroclor 1260	20 U	20 U	20 U	20 U	20 U	--	1.7 U	1.7 U	1.7 U	1.7 U	1.7 U	--	1.7 U	1.7 U	1.7 U	1.7 U	1.7 U	--
Total PCBs	20 U	20 U	20 U	**15** J	**12** J	**8.4**	1.7 U	1.7 U	1.7 U	1.7 U	1.7 U	--	1.7 U	1.7 U	1.7 U	1.7 U	1.7 U	--

Notes:
1. U = Not detected at the indicated method reporting limits (MRL).
2. J = Estimated concentration that is less than the MRL but greater than or equal to the MDL.
3. P = The GC or HPLC confirmation criteria were exceeded.
4. D = Reported result is from dilution.
5. NA = Not analyzed.
6. -- = Not applicable.
7. Mean = Mean tissue concentrations of constituents were calculated using one-half of the MDL for non-detected replicates (see laboratory certificates in Appendix D).

Table 12 - Tissue Results from Bioaccumulation Testing for *Corbicula fluminea*
Lower Willamette River Reference Area Study
Portland, Oregon

Sample ID	Day 0						Day 28 for Sediment Sample HC-08 Exposure					HC-08	Day 28 for Sediment Sample HC-10 Exposure					HC-10
Replicate Number	1	2	3	4	5	Day 0	1	2	3	4	5	Day 28	1	2	3	4	5	Day 28
Lab ID	K21108269-016	K21108269-017	K21108269-018	K21108269-019	K21108269-020	Mean	K2108883-006	K2108883-007	K2108883-008	K2108883-009	K2108883-010	Mean	K2108883-011	K2108883-012	K2108883-013	K2108883-014	K2108883-015	Mean
Conventionals as Wet Wt.																		
Sample Weight in grams	84.69	146.98	110.88	73.22	69.96	**97.15**	47.26	31.56	42.98	27.26	26.81	**35.17**	24.49	20.83	26.84	31.3	27.61	**26.21**
Total Lipids in %	0.95	0.61	0.8	0.82	1.11	**0.86**	0.47	0.85	0.73	0.73	0.99	**0.75**	0.83	0.83	1.11	0.78	1.05	**0.92**
Total Solids in %	9.6	7.4	8.5	10.4	10.9	**9.4**	8.37	11	9.34	10.4	12	**10.2**	11.6	13.5	11.8	11.2	12	**12.0**
Metals in mg/kg as Dry Wt																		
Antimony, Total	0.02 U	0.02 U	0.02 U	0.02 U	0.02 U	--	0.02 U	0.02 U	0.02 U	0.02 U	0.02 U	--	0.02 U	0.02 U	0.02 U	0.02 U	0.02 U	--
Arsenic, Total	**3.8**	**4**	**3.9**	**3.6**	**3.8**	**3.8**	**3.9**	**3.7**	**4**	**3.8**	**3.7**	**3.8**	**3.8**	**3.6**	**3.5**	**4**	**3.8**	**3.7**
Cadmium, Total	**0.56**	**0.61**	**0.59**	**0.56**	**0.54**	**0.57**	**0.59**	**0.5**	**0.56**	**0.51**	**0.5**	**0.5**	**0.52**	**0.49**	**0.43**	**0.56**	**0.49**	**0.50**
Chromium, Total	**1.4**	**3.2**	**0.8**	**1.1**	**1.2**	**1.5**	**1.4**	**0.9** J	**0.9** J	**1**	**1.1**	**1.1**	**0.9** J	**9.1**	**1**	**1.2**	**0.6** J	**2.6**
Copper, Total	**38**	**53.4**	**30.7**	**32.9**	**34.6**	**37.9**	**33.8**	**31.3**	**38**	**39.1**	**34.1**	**35.3**	**31.9**	**30.3**	**35.5**	**35**	**33.6**	**33.3**
Lead, Total	**0.3**	**0.66**	**0.33**	**0.27**	**0.32**	**0.38**	**0.19** J	**0.18** J	**0.19** J	**0.2** J	**0.17** J	**0.19**	**0.22** J	**0.17** J	**0.15** J	**0.19** J	**0.18** J	**0.18**
Mercury, Total	**0.23**	**0.24**	**0.22**	**0.23**	**0.23**	**0.23**	**0.2**	**0.18**	**0.19**	**0.21**	**0.19**	**0.19**	**0.21**	**0.18**	**0.19**	**0.2**	**0.22**	**0.2**
Nickel, Total	**1.7**	**10.4**	**1.29**	**1.33**	**1.65**	**3.27**	**1.3**	**1.3**	**1.3**	**1.2**	**0.8**	**1.2**	**1**	**4.8**	**0.8**	**1**	**0.9**	**1.7**
Silver, Total	**0.34**	**0.34**	**0.35**	**0.33**	**0.37**	**0.35**	**0.405**	**0.401**	**0.384**	**0.449**	**0.43**	**0.41**	**0.395**	**0.356**	**0.224**	**0.422**	**0.495**	**0.38**
Zinc, Total	**198**	**212**	**210**	**181**	**183**	**197**	**194**	**183**	**201**	**184**	**130**	**178.4**	**166**	**173**	**155**	**192**	**130**	**163.2**
Butyltins in µg/kg as Wet Wt.																		
Tetra-n-butyltin	1.0 U	1.0 U	1.0 U	1.0 U	1.0 U	--	1.0 U	1.5 U	1.0 U	2.0 U	2.0 U	--	2.0 U	2.1 U	2.0 U	2.0 U	2.1 U	--
Tri-n-butyltin (TBT)	**24**	**30**	**23**	**29**	**34**	**28**	**10**	**16**	**17**	**20**	**21**	**17**	**20**	**23**	**23**	**16**	**17**	**19.8**
Di-n-butyltin	**13**	**16**	**14**	**15**	**17**	**15**	**13**	**13**	**16**	**17**	**18**	**15**	**16**	**16**	**22**	**9.3**	**13**	**15.3**
n-Butyltin	**6.4**	**7.5**	**6.5**	**7.3**	**8.8**	**7.3**	**3.7**	**6.3**	**4.3**	**7.9**	**7.2**	**6**	**7.8**	**7.0**	**8.9**	**2.6** J	**6.3**	**6.5**
Semivolatiles in µg/kg as Wet Wt.																		
Acenaphthene	40 U	40 U	40 U	**12** J	40 U	**3**	na	**5.8** J	40 U	**2.6** J	73 U	**2.5**	80 U	80 U	73 U	54 U	73 U	--
Acenaphthylene	40 U	40 U	**1.9** J	**2.4** J	**2.9** J	**1.8**	na	**6.7** J	**2.5** J	**4** J	**3.2** J	**4**	**3** J	**3.6** J	**3.7** J	**2.6** J	**3.3** J	**3.2**
Anthracene	**3.9** J	**4** J	**3.8** J	**7.5** J	**6.5** J	**5.1**	na	**9.3** J	**5.4** J	**8.2** J	**6.4** J	**7**	**5.7** J	**6.6** J	**5.8** J	**5.3** J	**6.5** J	**6.0**
Fluorene	**3.1** J	40 U	40 U	**13** J	**5.3** J	**4.6**	na	**11** J	**5.3** J	**7.6** J	73 U	**6.2**	80 U	7.9 J	6.1 J	54 U	73 U	--
Naphthalene	**3.4** J	**3.6** J	**3.2** J	**9.8** J	**4.4** J	**4.9**	na	**11** J	**3.6** J	**7.6** J	**6.7** J	**7**	**6.7** J	**6.5** J	**6** J	**4.4** J	**6.2** J	**6.0**
Phenanthrene	**9.8** J	**10** J	**8.7** J	**33** J	**12** J	**14.7**	na	**16** J	**8.8** J	**13** J	**8.2** J	**12**	**7.5** J	**11** J	**12** J	**7.9** J	**9.3** J	**9.5**
2-Methylnaphthalene	40 U	40 U	40 U	**8.2** J	**3.6** J	**3.1**	na	**34** J	40 U	**4.1** J	**3.6** J	**10.7**	80 U	80 U	4.3 J	54 U	73 U	--
Benz(a)anthracene	40 U	40 U	40 U	40 U	40 U	--	na	**7.8** J	40 U	**11** J	73 U	**5.2**	80 U	80 U	73 U	54 U	73 U	--
Benzo(a)pyrene	40 U	40 U	40 U	40 U	40 U	--	na	62 U	40 U	**14** J	73 U	**4.3**	80 U	80 U	73 U	54 U	73 U	--
Benzo(b)fluoranthene	40 U	40 U	40 U	40 U	40 U	--	na	62 U	40 U	**17** J	73 U	**5**	80 U	80 U	73 U	54 U	73 U	--
Benzo(k)fluoranthene	40 U	40 U	40 U	40 U	40 U	--	na	62 U	40 U	**3.3** J	73 U	**1.7**	80 U	80 U	73 U	54 U	73 U	--
Benzo(g,h,i)perylene	40 U	40 U	40 U	40 U	40 U	--	na	62 U	40 U	73 U	73 U	**1.8**	80 U	80 U	73 U	54 U	73 U	--
Chrysene	40 U	40 U	40 U	40 U	40 U	--	na	**10** J	40 U	**12** J	73 U	**6**	80 U	80 U	73 U	54 U	73 U	--
Dibenz(a,h)anthracene	40 U	40 U	40 U	40 U	40 U	--	na	62 U	40 U	73 U	73 U	--	80 U	80 U	73 U	54 U	73 U	--
Fluoranthene	**14** J	**13** J	**12** J	**23** J	**17** J	**15.8**	na	**15** J	**13** J	**24** J	**13** J	**16**	**11** J	**13** J	**13** J	**11** J	**12** J	**12.0**
Indeno(1,2,3-cd)pyrene	40 U	40 U	40 U	40 U	40 U	--	na	62 U	40 U	**5.7** J	73 U	**2.1**	80 U	80 U	73 U	54 U	73 U	--
Pyrene	40 U	40 U	40 U	**17** J	40 U	**19.4**	na	**16** J	**12** J	**23** J	**14** J	**16**	80 U	**12** J	**13** J	540 U	730 U	**140.0**
Phenols in µg/kg as Wet Wt.																		
2,4-Dimethylphenol	80 U	80 U	80 U	80 U	80 U	--	na	130 U	80 U	150 U	150 U	--	160 U	160 U	150 U	110 U	150 U	--
2-Methylphenol	80 U	80 U	80 U	80 U	80 U	--	na	130 U	80 U	150 U	150 U	--	160 U	160 U	150 U	110 U	150 U	--
4-Methylphenol	80 U	80 U	80 U	80 U	80 U	--	na	130 U	80 U	150 U	150 U	--	160 U	160 U	150 U	110 U	150 U	--
Pentachlorophenol (PCP)	**230** J	**100** J	320 U	320 U	**140** J	**112**	na	500 U	320 U	590 U	590 U	--	640 U	640 U	590 U	430 U	590 U	--
Phenol	**160** J	**150** J	**160** J	**160** J	**190** J	**164**	na	**240** J	**170** J	**250** J	**280** J	**235**	**260** J	**280** J	**230** J	**170** J	**230** J	**234**
Misc. Semivolatiles in µg/kg as Wet Wt.																		
Benzoic Acid	**2700**	**1600** J	**1600**	**2400**	**2300**	**2120**	na	**2500**	1600 U	**1800** J	**3200**	**2062**	**3700**	**4500**	**2900** J	**2400**	**3200**	**3340**
Benzyl Alcohol	**82**	40 U	40 U	40 U	**50**	**35.1**	na	62 U	40 U	**54** J	73 U	**24.3**	80 U	80 U	73 U	54 U	73 U	--
Dibenzofuran	40 U	40 U	40 U	**10** J	40 U	**2.8**	na	**5.4** J	40 U	73 U	73 U	**2.1**	80 U	80 U	73 U	54 U	73 U	--
Hexachlorobutadiene	40 U	40 U	40 U	40 U	40 U	--	na	62 U	40 U	73 U	73 U	--	80 U	80 U	73 U	54 U	73 U	--
Hexachloroethane	40 U	40 U	40 U	40 U	40 U	--	na	62 U	40 U	**45** J	73 U	**24**	80 U	80 U	73 U	54 U	73 U	--
N-Nitrosodiphenylamine	80 U	80 U	80 U	80 U	80 U	--	na	130 U	80 U	150 U	150 U	--	160 U	160 U	150 U	110 U	150 U	--
1,2-Dichlorobenzene	40 U	40 U	40 U	40 U	40 U	--	na	62 U	40 U	73 U	73 U	--	80 U	80 U	73 U	54 U	73 U	--
1,3-Dichlorobenzene	40 U	40 U	40 U	40 U	40 U	--	na	62 U	40 U	73 U	73 U	--	80 U	80 U	73 U	54 U	73 U	--
1,4-Dichlorobenzene	40 U	40 U	40 U	40 U	40 U	--	na	62 U	40 U	73 U	73 U	--	80 U	80 U	73 U	54 U	73 U	--
1,2,4-Trichlorobenzene	40 U	40 U	40 U	40 U	40 U	--	na	62 U	40 U	73 U	73 U	--	80 U	80 U	73 U	54 U	73 U	--
Hexachlorobenzene	40 U	40 U	40 U	40 U	40 U	--	na	62 U	40 U	73 U	73 U	--	80 U	80 U	73 U	54 U	73 U	--

Please refer to notes at the end of this table.

Table 12 - Tissue Results from Bioaccumulation Testing for *Corbicula fluminea*
Lower Willamette River Reference Area Study
Portland, Oregon

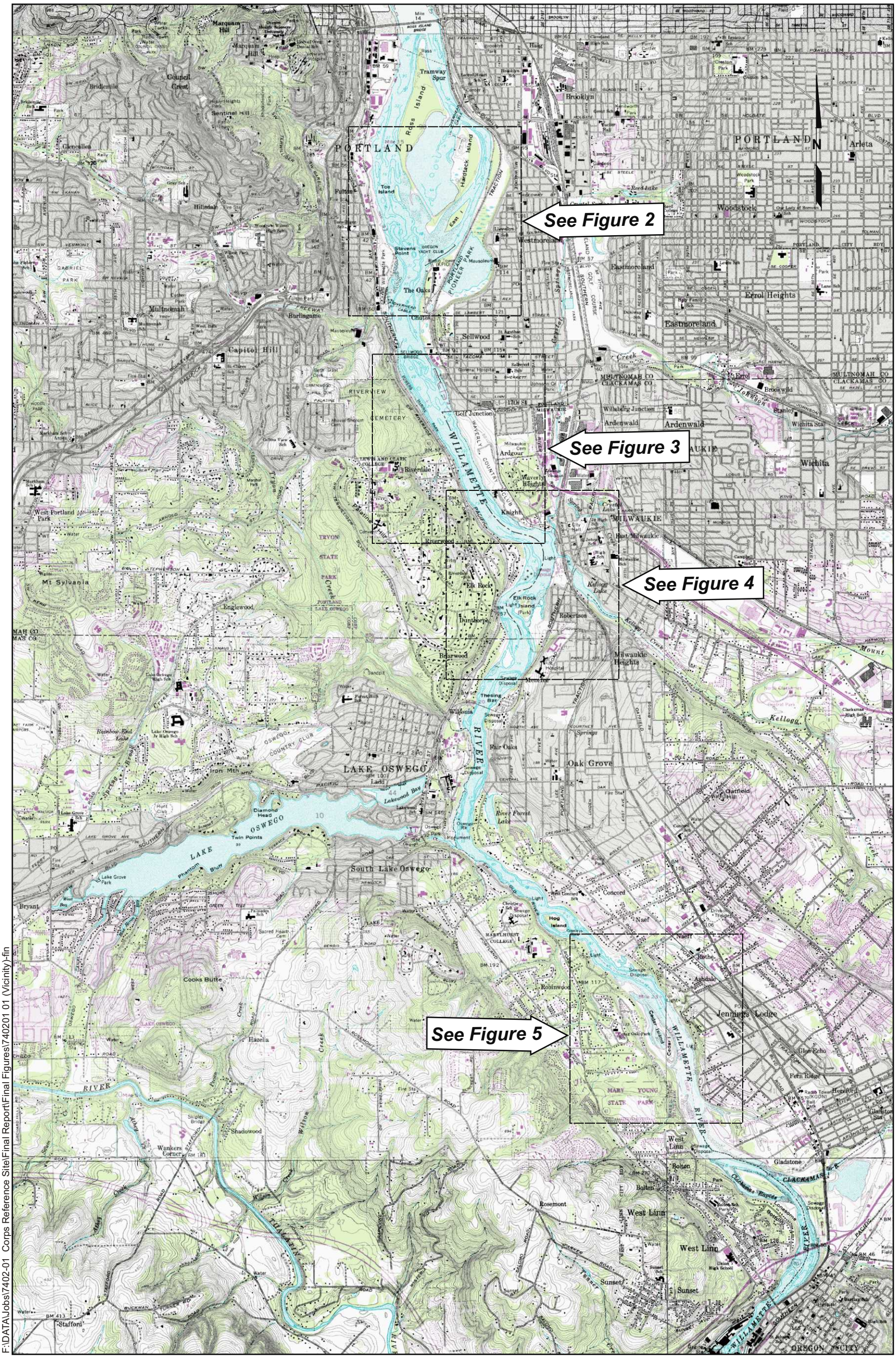
Sample ID	Day 0						Day 28 for Sediment Sample HC-08 Exposure					HC-08	Day 28 for Sediment Sample HC-10 Exposure					HC-10
Replicate Number	1	2	3	4	5	Day 0	1	2	3	4	5	Day 28	1	2	3	4	5	Day 28
Lab ID	K21108269-016	K21108269-017	K21108269-018	K21108269-019	K21108269-020	Mean	K2108883-006	K2108883-007	K2108883-008	K2108883-009	K2108883-010	Mean	K2108883-011	K2108883-012	K2108883-013	K2108883-014	K2108883-015	Mean
Phthalates in µg/kg																		
Bis(2-ethylhexyl) Phthalate	80 U	80 U	**160**	**290**	**62** J	**113.8**	na	**82** J	**66** J	**94** J	**87** J	**82**	**100** J	**110** J	**110** J	**120**	**89** J	**106**
Butyl Benzyl Phthalate	40 U	40 U	40 U	40 U	40 U	--	na	62 U	40 U	73 U	73 U	--	80 U	80 U	73 U	54 U	73 U	--
Di-n-butyl Phthalate	40 U	40 U	40 U	40 U	40 U	--	na	62 U	40 U	**56** J	73 U	**25.3**	80 U	80 U	73 U	54 U	73 U	--
Di-n-octyl Phthalate	40 U	40 U	40 U	40 U	40 U	--	na	62 U	40 U	73 U	73 U	--	80 U	80 U	73 U	54 U	73 U	--
Diethyl Phthalate	**29** J	**26** J	**29** J	**29** J	**39** J	**30.4**	na	**31** J	40 U	**27** J	**32** J	**27.8**	80 U	**24** J	**31** J	54 U	73 U	**17.3**
Dimethyl Phthalate	40 U	40 U	40 U	40 U	40 U	--	na	62 U	40 U	73 U	73 U	--	80 U	80 U	73 U	54 U	73 U	--
Pesticides/PCBs in µg/kg as Wet Wt.																		
4,4'-DDD	1 U	1 U	1 U	1 U	1 U	--	na	1.6 U	1 U	1.9 U	1.9 U	--	2 U	2 U	1.9 U	1.4 U	1.9 U	--
4,4'-DDE	**0.57** J	**0.74** J	**0.8** J	**0.58** J	**0.71** J	**0.68**	na	**1.4** JP	**0.93** J	1.9 U	**1.5** J	**1.2**	**2.1** P	**1.3** JP	**1.2** J	**0.74** J	**1.9**	**1.4**
4,4'-DDT	1 U	1 U	1 U	2.4 U	1 U	--	na	1.6 U	1 U	1.9 U	1.9 U	--	2 U	2 U	1.9 U	1.4 U	1.9 U	--
Total DDT	**0.57** J	**0.74** J	**0.8** J	**0.58** J	**0.71** J	**0.68**	na	**1.4** JP	**0.93** J	1.9 U	**1.5** J	**1.2**	**2.1** P	**1.3** JP	**1.2** J	**0.74** J	**1.9**	**1.4**
Aldrin	1 U	1 U	1 U	1 U	1 U	--	na	1.6 U	1 U	1.9 U	1.9 U	--	2 U	2 U	1.9 U	1.4 U	1.9 U	--
alpha-Chlordane	**1.5**	**1.6** P	**1.9**	**1.4**	**1.6**	**1.6**	na	**1.5** J	**1.5**	**2**	**2.2**	**1.80**	**1.8** J	**2** J	**2.2**	**1.6**	**2**	**1.9**
gamma-Chlordane	**0.8** J	**0.66** JP	**0.79** JP	**0.91** J	**0.89** J	**0.81**	na	**1.1** J	**0.97** J	**1.3** J	**1.3** J	**1.17**	**1.5** J	**1.3** J	**1.4** J	**1** J	**1.3** JP	**1.3**
Dieldrin	1 U	1 U	1.3 U	19 U	1 U	--	na	20 U	1 U	17 U	22 U	--	19 U	2 U	1.9 U	19 U	19 U	--
Heptachlor	1 U	1 U	1 U	1 U	1 U	--	na	1.6 U	1 U	1.9 U	1.9 U	--	2 U	2 U	1.9 U	1.4 U	1.9 U	--
gamma-BHC (Lindane)	1 U	1 U	1 U	1 U	1 U	--	na	1.6 U	1 U	1.9 U	1.9 U	--	2 U	2 U	1.9 U	1.4 U	1.9 U	--
Aroclor 1016	10 U	10 U	10 U	10 U	10 U	--	na	16 U	10 U	19 U	19 U	--	20 U	20 U	19 U	14 U	19 U	--
Aroclor 1221	20 U	20 U	20 U	20 U	20 U	--	na	31 U	20 U	37 U	37 U	--	40 U	40 U	37 U	27 U	37 U	--
Aroclor 1232	10 U	10 U	10 U	10 U	10 U	--	na	16 U	10 U	19 U	19 U	--	20 U	20 U	19 U	14 U	19 U	--
Aroclor 1242	10 U	10 U	10 U	10 U	10 U	--	na	16 U	10 U	19 U	19 U	--	20 U	20 U	19 U	14 U	19 U	--
Aroclor 1248	10 U	10 U	10 U	10 U	10 U	--	na	16 U	10 U	19 U	19 U	--	20 U	20 U	19 U	14 U	19 U	--
Aroclor 1254	10 U	10 U	10 U	10 U	10 U	--	na	16 U	10 U	19 U	19 U	--	20 U	20 U	19 U	14 U	19 U	--
Aroclor 1260	10 U	10 U	10 U	10 U	10 U	--	na	**13** J	10 U	19 U	19 U	**6.2**	20 U	20 U	19 U	14 U	**14** J	**6.4**
Total PCBs	20 U	10 U	10 U	10 U	10 U	--	na	**13** J	20 U	37 U	37 U	**6.2**	40 U	40 U	37 U	27 U	**14** J	**6.4**

F:\DATA\Jobs\7402-01 Corps Reference Site\Final Report\Tables\Tables 11-12

Notes:

1. U = Not detected at the indicated method reporting limits (MRL).
2. J = Estimated concentration that is less than the MRL but greater than or equal to the MDL.
3. P = The GC or HPLC confirmation criteria were exceeded.
4. D = Reported result is from dilution.
5. NA = Not analyzed.
6. -- = Not applicable.
7. Mean = Mean tissue concentrations of constituents were calculated using one-half of the MDL for non-detected replicates (see laboratory certificates in Appendix D).

Site and Vicinity Plan
Phase I and Phase II Sediment Sample Locations
Lower Willamette River Reference Area Study



F:\DATA\Jobs\7402-01 Corps Reference Site\Final Report\Final Figures\7402-01 01 (Vicinity)-fin

Note: Base map prepared from the USGS 7.5-minute quadrangle of Lake Oswego, Oregon City, Gladstone, Canby, and Portland, Oregon, dated 1990.

0 4,000 8,000

Approximate Scale in Feet

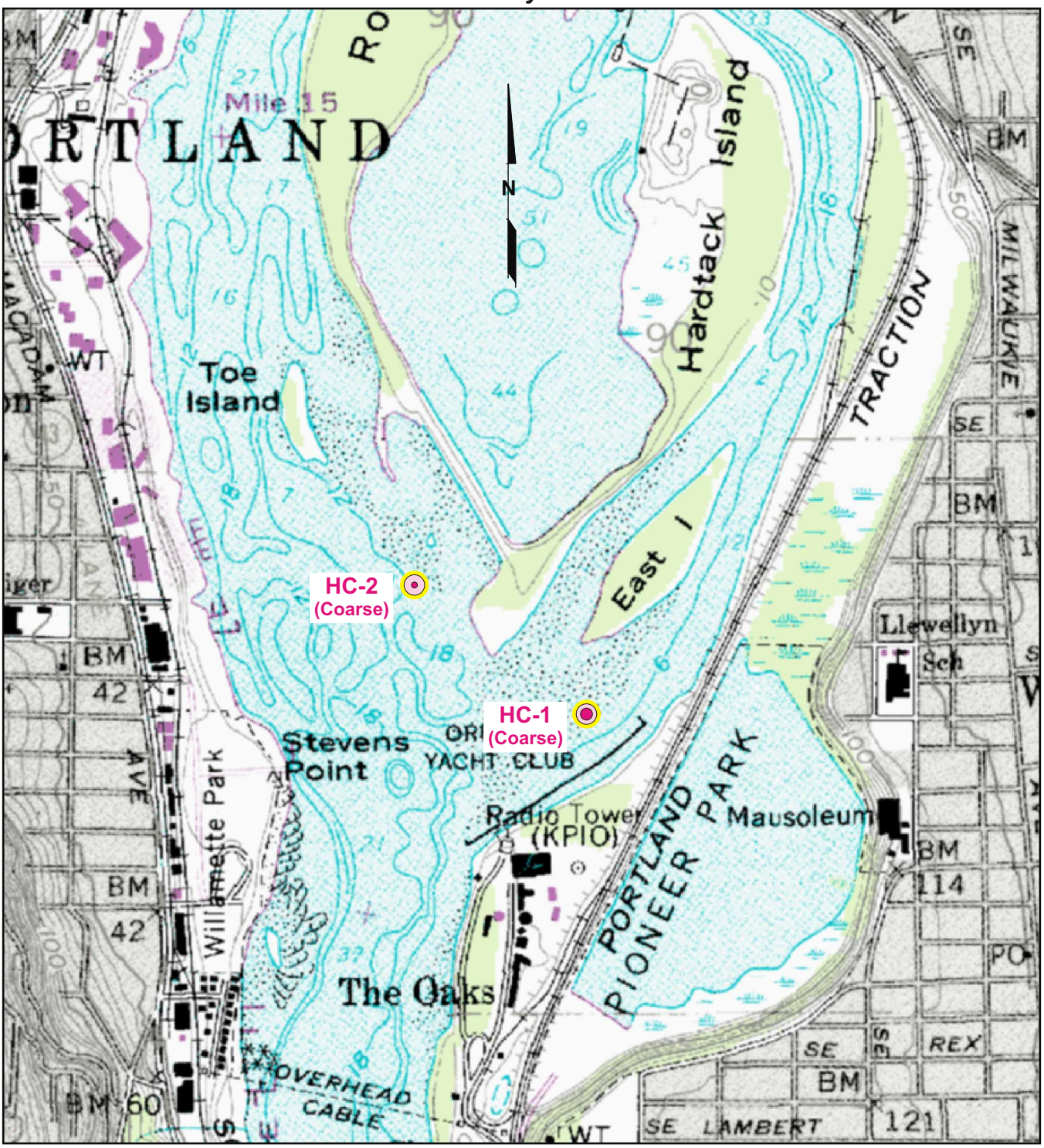
HARTCROWSER

7402-01

Figure 1

4/02

Site Plan #1
Phase I and Phase II Sediment Sample Locations
Lower Willamette River Reference Area Study



F:\DATA\Jobs\7402-01 Corps Reference Site\Final Report\Final Figures\740201 02 (Site 1)-fin

Note: Base map prepared from the USGS 7.5-minute quadrangle of Lake Oswego, Oregon, dated 1990.

Legend:

HC-1 (Coarse) — Phase I Sediment Grab Sample Location and Number (Target Grain Size Range)

HC-2 (Coarse) — Phase I and II Sediment Grab Sample Location and Number (Target Grain Size Range)

0 1,000 2,000

Approximate Scale in Feet

HARTCROWSER

7402-01 ***4/02***

Figure 2

Site Plan #2
Phase I and Phase II Sediment Sample Locations
Lower Willamette River Reference Area Study

F:\DATA\Jobs\7402-01 Corps Reference Site\Final Report\Final Figures\740201 03 (Site 2)-fin

Note: Base map prepared from the USGS 7.5-minute quadrangle of Lake Oswego and Gladstone, Oregon, dated 1990.

0 1,000 2,000

Approximate Scale in Feet

Legend:

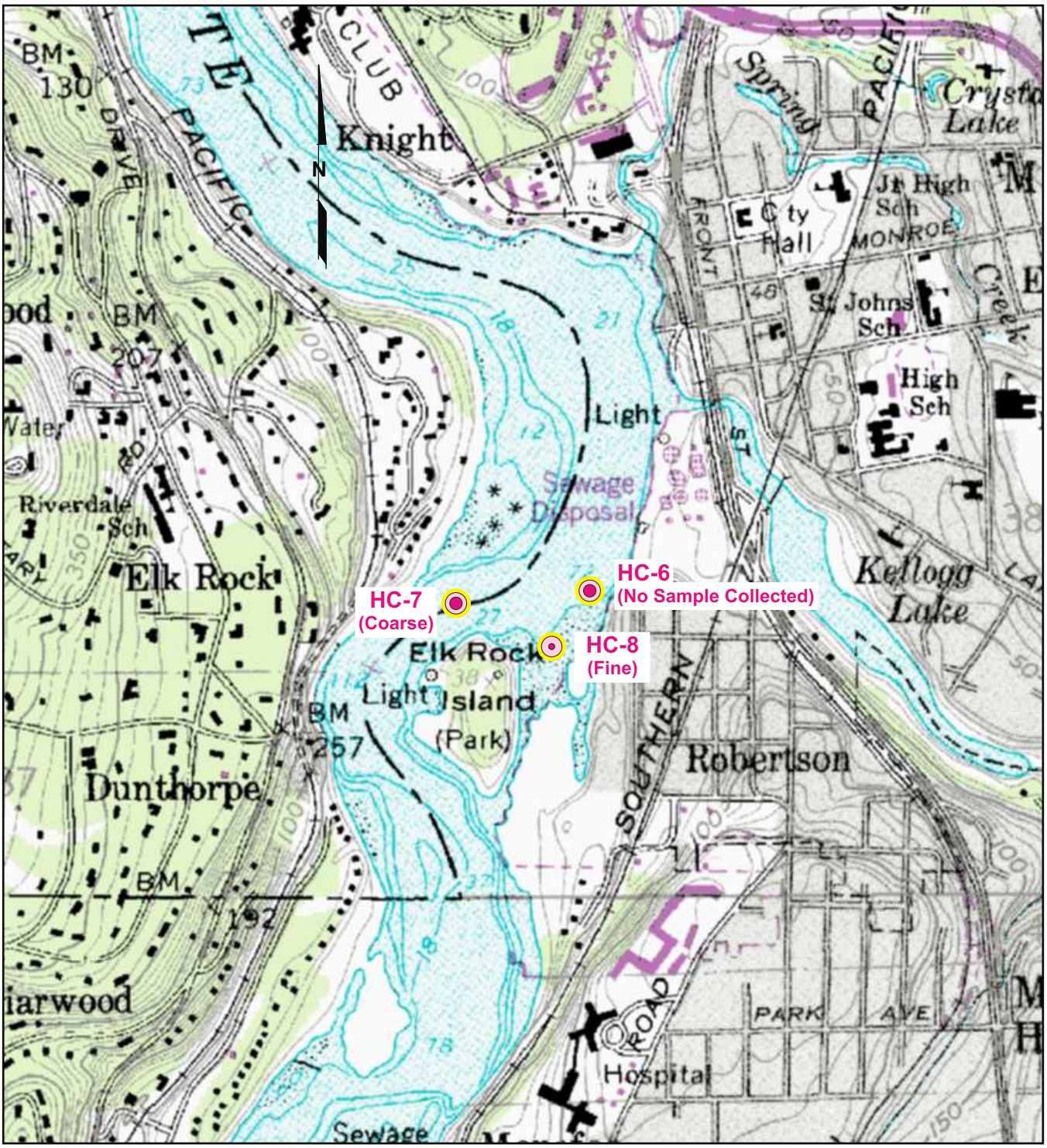
HC-4 (Fine) Phase I Sediment Grab Sample Location and Number (Target Grain Size Range)

HARTCROWSER

7402-01 ***4/02***

Figure 3

Site Plan #3
Phase I and Phase II Sediment Sample Locations
Lower Willamette River Reference Area Study



F:\DATA\Jobs\7402-01 Corps Reference Site\Final Report\Final Figures\740201 04 (Site 3)-fin

Note: Base map prepared from the USGS 7.5-minute quadrangle of Lake Oswego, Oregon, dated 1990.

Legend:

HC-7 (Coarse) — Phase I Sediment Grab Sample Location and Number (Target Grain Size Range)

HC-8 (Fine) — Phase I and II Sediment Grab Sample Location and Number (Target Grain Size Range)

0 1,000 2,000

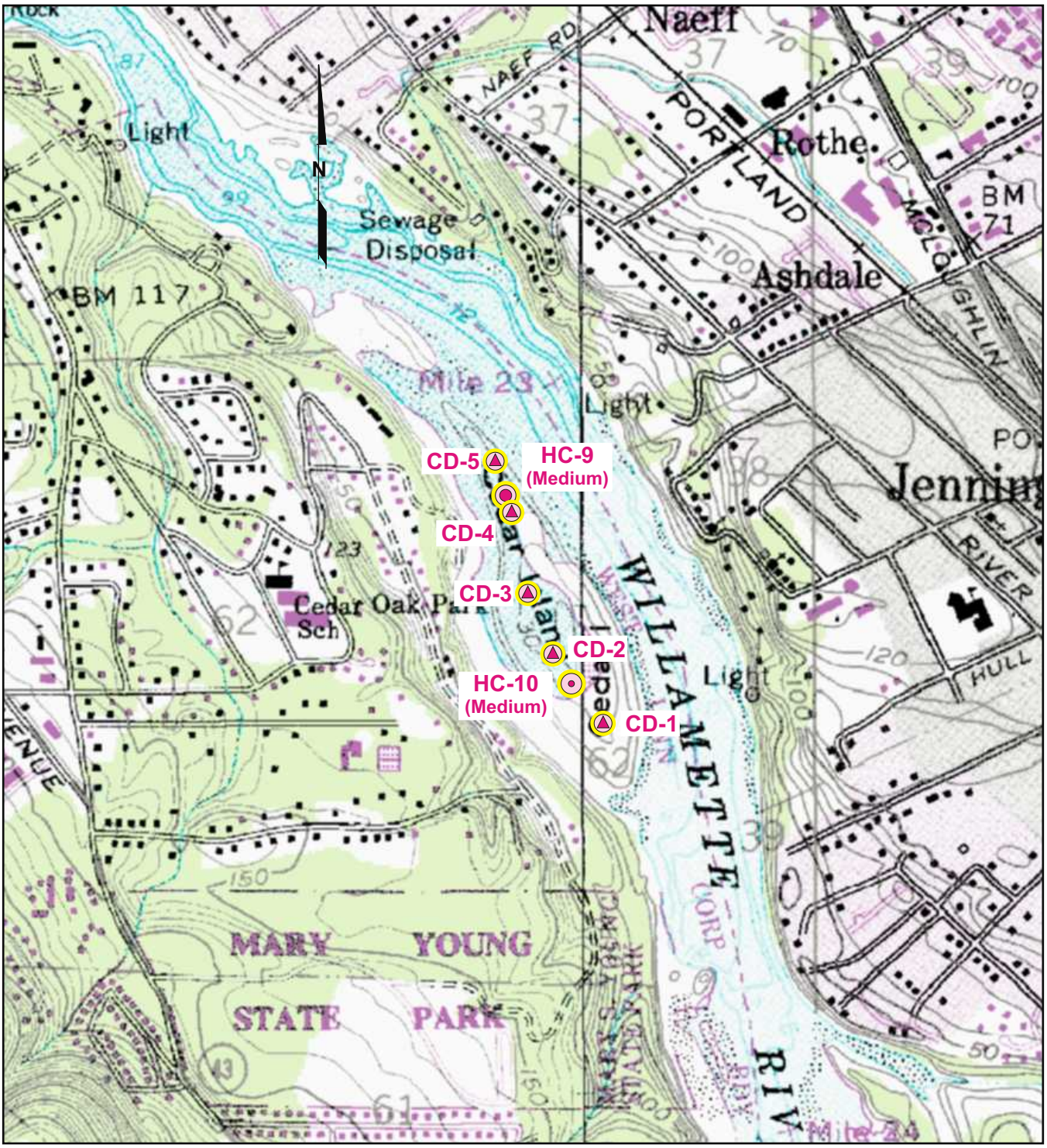
Approximate Scale in Feet

HARTCROWSER

7402-01 ***4/02***

Figure 4

Site Plan #4
Phase I and Phase II Sediment Sample Locations
Lower Willamette River Reference Area Study



F:\DATA\Jobs\7402-01 Corps Reference Site\Final Report\Final Figures\740201 02 (Site 1)-fin\740201 05 (Site 4)-fin

Note: Base map prepared from the USGS 7.5-minute quadrangle of Lake Oswego, Oregon, dated 1990.

Legend:

HC-9 (Medium) — Phase I Sediment Grab Sample Location and Number (Target Grain Size Range)

HC-10 (Medium) — Phase I and II Sediment Grab Sample Location and Number (Target Grain Size Range)

CD-1 — Phase II Sediment Grab Sample (Analyzed for Grain Size, TVS, and TOC Only)

0 1,000 2,000

Approximate Scale in Feet

HARTCROWSER

7402-01 ***4/02***

Figure 5

APPENDIX A
PHOTOGRAPH LOG

Photograph 1 - Cedar Island sample location HC-10 facing south.

Photograph 2 - Cedar Island sample location HC-10 facing west.

Photograph 3 - Cedar Island sample location HC-10 facing north.

Photograph 4 - Van Veen sediment grab sampler.

Photograph 5 - Elk Rock Island sample location HC-7 facing northeast.

Photograph 6 - Willamette River north of Elk Rock Island facing east.

Photograph 7 - Willamette River facing south viewing Elk Rock Island.

Photograph 8 - Willamette River sample location HC-3 facing southwest.

Photograph 9 - Willamette River sample location HC-3 facing northwest.

Photograph 10 - Willamette River below Sellwood Bridge facing southwest.

Photograph 11 - Willamette River sample location HC-2 near Ross Island facing east.

Photograph 12 - Willamette River sample location HC-1 near Ross Island facing west.

Photograph 13 - Wet sieving field method at sample location HC-1.

Photograph 14 - Air-powered van Veen sediment sampler before deployment.

Photograph 15 - Air-powered van Veen sediment sampler after deployment.

APPENDIX B
BIOASSAY AND BIOACCUMULATION TESTING PROTOCOLS AND REPORTS

TOXICITY TEST REPORT

TEST IDENTIFICATION

Test No.: 645-1

Title: Toxicity of freshwater sediments using a 10-day midge, *Chironomus tentans*, sediment bioassay.

Protocol No.: NAS-XXX-CT4b, April 7, 1998. Based on ASTM 1996 (Standard test methods for measuring the toxicity of sediment-associated contaminants with fresh water invertebrates, E1706-95b), Am. Soc. Test. Mat., Phila., PA, and EPA Method 100.2 (Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates, EPA/600/R-94/024).

STUDY MANAGEMENT

Study Sponsor: Hart Crowser, Inc., 5 Center Pointe Dr., Suite 240, Lake Oswego, Oregon 97035

Sponsor's Study Monitor: Taku Fuji

Testing Laboratory: Northwestern Aquatic Sciences, P.O. Box 1437, Newport, OR 97365

Test Location: Newport laboratory

Laboratory's Study Personnel: G.J. Irissarri, B.S., Proj. Man./Study Dir.; L.K. Nemeth, M.B.A., QA Officer; R.J. Caldwell, PhD, Senior Toxicologist; G.A. Buhler, B.S., Aq. Toxicologist; G. Hayes, B.S., Tech.

Study Schedule:

Test Beginning: 10-2-01, 1300 hrs.

Test Ending: 10-12-01, 1200 hrs.

Disposition of Study Records: All specimens, raw data, reports and other study records are stored according to Good Laboratory Practice regulations at Northwestern Aquatic Sciences, 3814 Yaquina Bay Rd., Newport, OR 97365.

Good Laboratory Practices: The test was conducted following the principles of Good Laboratory Practices (GLP) as defined in the EPA/TSCA Good Laboratory Practice regulations revised August 17, 1989 (40 CFR Part 792).

Statement of Quality Assurance: The test data were reviewed by the Quality Assurance Unit to assure that the study was performed in accordance with the protocol and standard operating procedures. This report is an accurate reflection of the raw data.

TEST MATERIAL

Test Sediments: Unidentified freshwater test sediments. Details are as follows:

NAS Sample No.	7576F	7577F	7578F
Description	HC-02	HC-08	HC-10
Collection Date	9-17-01	9-17-01	9-17-01
Receipt Date	9-18-01	9-18-01	9-18-01

Control Sediment: The negative control sediment (NAS#7579F) was collected on 9-18-01 from an area approximately one mile east of the Hwy. 101 bridge at Beaver Creek, approx. 8 miles south of Newport, OR. The control sediment was press sieved through a 0.5 mm screen.

Storage: All test and reference sediments were stored at 4°C in the dark until used.

TEST WATER

Source: Moderately hard synthetic water prepared from Milli-Q® deionized water.

Dates of Preparation: 9-28-01, 10-3-01, 10-8-01

Water Quality: pH 8.1, 8.1, 8.0; conductivity 300, 340, 320 µmhos/cm; hardness 85, 94, 94 mg/L as $CaCO_3$; alkalinity 70, 80, 80mg/L as $CaCO_3$.

Pretreatment: Aerated ≥24 hr.

TEST ORGANISMS

Species: *Chironomus tentans*, midge.
Age/Size: 3rd instar, 0.35 mm average head capsule width
Source: NAS cultures, originally obtained from EPA, Duluth, MN.
Acclimation: Temperature, 23.0 ± 1.0°C; dissolved oxygen, 7.1 ± 0.5 mg/L; pH, 7.9 ± 0.3; conductivity, 345 ± 46 µmhos/cm; hardness, 102 mg/L as $CaCO_3$; and alkalinity, 100 mg/L as $CaCO_3$.

TEST PROCEDURES AND CONDITIONS

The following is an abbreviated statement of the test procedures and a statement of the test conditions actually employed. See the test protocol (Appendix I) for a more detailed description of the test procedures used in this study.

Test Chambers: 300 ml high-form glass beakers
Test Volumes: 100 ml sediment layer; 175 ml test water.
Replicates/Treatment: 8
Organisms/Treatment: 80
Water Volume Changes: 2 water volumes per day
Aeration: None.
Feeding: Animals were fed 1.5 ml of TetraFin suspension (1.5 ml contains 6 mg dry solids) per beaker daily.
Effects Criteria: 1) survival after 10 days, and 2) average individual biomass (based on dry weight) after 10 days. Death is defined as no visible movement or response to tactile stimulation. Missing organisms were considered to be dead.
Water Quality and Other Test Conditions: The temperature, dissolved oxygen, conductivity, pH, hardness, alkalinity and ammonia-nitrogen were measured in the overlying water of one replicate test container per treatment on days 0 and 10 of the test. Temperature and dissolved oxygen were measured daily in the overlying water of one replicate test container per treatment. Hardness and alkalinity were measured with titrimetric methods. Ammonia-N was measured using Hach test kits based on the salicylate (Clin. Chim. Acta 14:403, 1996) colorimetric method; samples were not distilled prior to analysis. The photoperiod was 16:8, L:D.

DATA ANALYSIS METHODS

Survival and individual biomass were calculated for each replicate as follows:

percent survival = 100 x (number surviving/initial number tested)
average individual ash-free biomass = (final wt. – ashed dry wt.)/number weighed,
where:
ash-free dry wt. = dry weight of organisms recovered on day 20 – ashed dry weight, in mg

Means and standard deviations for the biological endpoints described above, and for water quality data, were computed using Microsoft Excel 2000. The value for mortality and individual biomass for each test sediment was statistically compared against the control sediment. An arcsine square root transformation was performed on proportional mortality data before analysis. Following determination of normality and homogeneity of variances, a one-tailed Student T-test, Mann-Whitney or Approximate T test was conducted at the 0.05 level of significance. The statistical software used was BioStat (Beta v.2.0c) bioassay software developed by the U.S. Army Corps of Engineers, Seattle District.

PROTOCOL DEVIATIONS

None

REFERENCE TOXICANT TEST

The reference toxicant test is a multi-concentration toxicity test using potassium chloride, to evaluate the performance of the test organisms used in the sediment toxicity test. The performance is evaluated by comparing the results of this test with historical results obtained at the laboratory. A summary of the reference toxicant test result is given below. The reference toxicant test raw data are found in Appendix III.

Test No.: 999-1329
Reference Toxicant and Source: Potassium chloride (Mallinckrodt, Lot No. 6845 KXAT).
Test Date: 10-2-01
Dilution Water Used: Moderately hard synthetic water prepared from Milli-Q® deionized water.
Result: 96-hr LC50, 4.55 g/L. This result is within the laboratory's control chart warning limits (2.95 to 7.22 g/L).

TEST RESULTS

Observations of water quality in the overlying water throughout the test are summarized in Table 1. A detailed tabulation of the water quality results by sample and test day can be found in Appendix II. Interstitial ammonia and sulfide measurements are listed in Table 2. The means and standard deviations of percent mortality and growth (weight) of midges exposed for 10 days to sediments are summarized in Table 3. Detailed data organized by sample and replicate, and summary statistics for these observations, are given in Appendix II.

All water quality observations of overlying water temperature and dissolved oxygen were within the protocol specified ranges. Ammonia-N in the overlying water was <0.5 mg/L in all day 0 observations. On day 10, overlying water ammonia-N measured <0.5 mg/L except for HC-10 and the control, which contained 0.5 mg/L.

The test met acceptability criteria specified in the protocol with 81.3% mean control survival (70% required) and 0.72 mg average individual control weight (0.48 mg required). The reference toxicant (positive control) result was within the laboratory's control chart limits (4.55 g/L; control chart mean ± 2 S.D. = 5.09 ± 2.14).

None of the sediments tested produced mortality or biomass results that were found to be statistically significantly different from that of the control sediment.

STUDY APPROVAL

_____		_____	
Project Manager/Study Director	Date	Quality Assurance Unit	Date

_____

Laboratory Director Date

Table 1. Summary of water quality conditions during tests of the midge, *Chironomus tentans*, exposed to freshwater sediments.

Water Quality Parameter	Mean ± S.D.	Minimum	Maximum	N
Temperature (°C)	22.8 ± 0.2	22.5	23.4	44
Dissolved oxygen (mg/L)	5.4 ± 1.7	2.6	8.0	44
Conductivity (µmhos/cm)	331 ± 37	310	420	8
PH	7.7 ± 0.3	7.4	8.0	8
Hardness (mg/L as $CaCO_3$)	92 ± 6	85	102	8
Alkalinity (mg/L as $CaCO_3$)	79 ± 4	70	80	8
Sulfides (mg/L)	<0.02	<0.02	<0.02	8
Total ammonia (mg/L)	<0.5	<0.5	0.5	8

Table 2. Interstitial ammonia and pH measurements of test sediments used in test.

Sample description	Ammonia (mg/L)	pH
Control (NAS#7579F)	---	---
HC-02 (NAS#7576F)	<0.05	6.7
HC-08 (NAS#7577F)	1.5	6.9
HC-10 (NAS#7578F)	1.5	6.9

Table 3. Survival and growth (ash-free dry weight) of *Chironomus tentans* exposed for 10 days to freshwater sediments. All statistical comparisons were made against the control at the 0.05 level of significance.

Sample description	Percent mortality (Mean ± SD)	Statistically significantly different?	Average wt/midge (mg) [1] (Mean ± SD)	Statistically significantly different?
Control (NAS#7579F)	18.8 ± 16.4	---	0.72 ± 0.09	---
HC-02 (NAS#7576F)	13.8 ± 9.2	No	0.77 ± 0.10	No
HC-08 (NAS#7577F)	7.5 ± 7.1	No	0.62 ± 0.05	No
HC-10 (NAS#7578F)	16.3 ± 16.0	No	0.75 ± 0.11	No

[1] Pupae were not included in the sample to estimate ash-free dry weight (as per EPA/600/R-94/024, p. 55, section 12.3.8.1)

APPENDIX I

PROTOCOL

TEST PROTOCOL

FRESHWATER MIDGE, *CHIRONOMUS TENTANS*, 10-DAY SEDIMENT TOXICITY TEST

1. INTRODUCTION

1.1 Purpose of Study: The purpose of this study is to characterize the toxicity of freshwater sediments based on midge survival and growth using the midge, *Chironomus tentans*.

1.2 Referenced Method: This protocol is based on ASTM Method E 1706-95b (ASTM 1996) and EPA Method 100.2 (EPA/600/R-94/024).

1.3 Summary of Method: A summary of test conditions for the midge 10-day sediment toxicity test is tabulated below. The 10-day sediment toxicity test with Chironomus tentans is conducted at 23°C with a 16L:8D photoperiod at an illuminance of about 50-100 footcandles. Test chambers are 300-mL high-form lipless beakers containing 100 mL of sediment and 175 mL of overlying water. Ten third-instar midges are used in each replicate (all organisms must be third instar or younger and at least 50% of the larvae must be third instar). The number of replicates/treatment depends on the objective of the test. Eight replicates are recommended for routine testing. Midges in each test chamber are fed 1.5 mL of a 4 g/L fish food flakes suspension daily. Each chamber receives two volume additions per day of overlying water. Overlying water can be culture water, well water, surface water, site water, or reconstituted water. Test endpoints include survival and/or growth.

1. Test type	whole sediment toxicity test with renewal of overlying water
2. Test duration	10 days
3. Temperature	23 ± 1°C
4. Light quality	daylight flourescent light
5. Illuminance	50-100 footcandles
6. Photoperiod	16L:8D
7. Test chamber size	300-mL high-form lipless beakers (Pyrex® 1040 or equivalent)
8. Sediment volume	100 mL
9. Overlying water volume	175 mL
10. Renewal overlying water	2 volume additions/day (continuous or intermittent)
11. Age of test organisms	3rd instar or younger larvae (≥ 50% of organisms must be 3rd instar)
12. Organisms per test chamber	10
13. Replicates per treatment	8 recommended for routine (depends on design)
14. Organisms per treatment	80
15. Feeding regime	Fish food flakes, fed 1.5 mL daily/chamber (1.5 mL contains 6.0 mg of dry solids)
16. Aeration	None, unless DO falls below 40% saturation
17. Overlying (test) water	**Culture water**, well water, surface water, site water or reconstituted water
18. Water quality	Hardness, alkalinity, conductivity, pH, ammonia-N beginning and end; temperature and dissolved oxygen daily
19. Endpoints	Survival and growth (dry weight)
20. Test acceptability criteria	Minimum control survival of 70%; mean weight of surviving control organisms 0.6 mg
21. Sample holding	14 days at 4°C in the dark
22. Sample volume required	1L (800 mL per sediment)
23. Reference toxicant	Concurrent testing required

2. STUDY MANAGEMENT

2.1 Sponsor's Name and Address:

_____
_____
_____
_____

2.2 Sponsor's Study Monitor:

_____

2.3 Name of Testing Laboratory:
Northwestern Aquatic Sciences
3814 Yaquina Bay Road, P.O. Box 1437
Newport, OR 97365.

2.4 Test Location: _____

2.5 Laboratory's Personnel to be Assigned to the Study:
Study Director: _____
Quality Assurance Unit: _____
Aquatic Toxicologist: _____
Aquatic Toxicologist: _____

2.6 Proposed Testing Schedule: Tests are to begin within 14 days of sample collection. Reference toxicant test to be run concurrently.

2.7 Good Laboratory Practices: The test is conducted following the principles of Good Laboratory Practices (GLP) as defined in the EPA/TSCA Good Laboratory Practice regulations revised August 17, 1989 (40 CFR Part 792).

3. TEST MATERIAL

The test materials are freshwater sediments. The control, reference, and test sediments are placed in solvent cleaned 1 L glass jars fitted with PTFE-lined screw caps. At the laboratory the samples are stored at 4°C in the dark. The original sealed containers may be stored for up to 14 days prior to testing. If jars are not full when received or if sediment is removed for testing, headspaces should be filled with nitrogen toretard deterioration. A negative control sediment is collected from a clean site. In addition, a reference sediment, a clean sediment with physical characteristics similar to the test sediments, may be employed as a comparison station.

4. TEST WATER

Test water (overlying water) at NAS is normally *C. tentans* culture water, which is moderately hard synthetic water at a hardness of 80-100 mg/L as $CaCO_3$ and alkalinity of 60-70 mg/L as $CaCO_3$. Dilution water is prepared from Milli-Q reagent grade water and reagent grade chemicals. Test water may also be well water, surface water or site water depending on the study design. Current research is being done by EPA on a new type of reconstituted water that may be used for this test in the future (STM 1996, EPA 1994).

5. TEST ORGANISMS

5.1 Species: midge, *Chironomus tentans*.

5.2. Source: Cultured at NAS. Originally obtained from U.S. EPA Environmental Research Lab, Duluth, MN.

5.3 Age: Third instar or younger larvae (at least 50% of the larvae must be in the third instar at the start of the test). Third instar is normally 8.5 to 12.5 days after hatching; head capsule widths range from 0.33 to 0.45 mm.

5.4 Acclimation and Pretest Observation: Cultures are maintained at 23 ± 1°C under a 16:8 L:D photoperiod. The culture water is moderately hard synthetic water. Midge are fed finely ground Tetrafin flakes in suspension (10g Tetrafin in 100 mL Milli-Q water). Mortality during the 48-hr prior to testing should not be excessive.

6. DESCRIPTION OF TEST SYSTEM

6.1 Test Chambers and Environmental Control: Test chambers used in the toxicity test are 300-mL high-form lipless glass beakers (Pyrex® 1040 or equivalent). Test chambers are maintained at constant temperature by partial immersion in a temperature-controlled water bath or by placement in a temperature-controlled room. Aeration is not empolyed unless dissolved oxygen drops below 40% saturation. The test is conducted under an illuminance of 50-100 footcandles with a 16L:8D photoperiod.

6.2 Cleaning: All laboratory glassware, including test chambers, is cleaned as described in EPA/600/4-90/027F. New glassware and test systems are soaked 15 minutes in tap water and scrubbed with detergent (or cleaned in automatic dishwasher); rinsed twice with tap water; carefully rinsed once with fresh, dilute (10%, V:V) hydrochloric or nitric acid to remove scale, metals, and bases; rinsed twice with deionized water; rinsed once with acetone to remove organic compounds (using a fume hood or canopy); and rinsed three times with deionized water. Test systems and chambers are rinsed again with dilution water just before use.

7. EXPERIMENTAL DESIGN AND TEST PROCEDURES

7.1 Experimental Design: The test involves exposure of midge larvae to test, control, and reference sediments. The sediments are placed on the bottom of the test containers and are overlain with test water. The test exposure is for 10 days. The renewal of overlying water consists of two volume additions per day, either continuous or intermittent. Each treatment consists of eight replicate test containers, each containing 10 organisms. Test chamber positions are completely randomized. Test organisms are randomly distributed to the test chambers. Blind testing is normally used.

7.2 Setup of Test Containers: Sediments are homogenized and placed in test chambers on the day before addition of test organisms. Sediment (100 ml) is placed into each of eight replicate beakers. After addition of the sediment, 175 ml of test water is gently added to each beaker in a manner to prevent resuspension. The overlying water is replaced twice daily. The test begins when midges are introduced to the test chambers. Initial water quality measurements are taken prior to the addition of test organisms.

7.3 Effect Criterion: The acute effect criterion used in the midge bioassay is mortality, defined as the lack of movement of body or appendages on response to tactile stimulation. The optional chronic effect criterion is growth which is determined by using dry weight measurements.

7.4 Test Conditions: No aeration is employed unless dissoved oxygen falls below 40% saturation. The test temperature employed is 23°C (range of ± 1°C). A 16:8, L:D photoperiod is used. Illumination is supplied by daylight fluorescent lamps at 50-100 footcandles. The overlying water is replaced twice daily.

7.5 Beginning the Test: The test is begun by adding the organisms to the equilibrated test containers as previously described. If the optional growth endpoint is to be used, three extra replicates of midge larvae should be counted out and randomly selected for drying to determine initial average weight data.

7.6 Feeding: Midge larvae are fed 1.5 mL daily per test chamber (1.5 mL contains 6.0 mg of dry solids). A feeding may be skipped if there is a build up of excess food. However, all beakers must be treated similarly.

7.7 Test Duration, Type and Frequency of Observations, and Methods: The duration of the acute toxicity test is 10 days. The type and frequency of observations to be made are summarized as follows:

TYPE OF OBSERVATION	TIMES OF OBSERVATION (ASTM 1996)
BIOLOGICAL DATA	
Survival, growth	Day 10
PHYSICAL AND CHEMICAL DATA	
Hardness, alkalinity, ammonia-N, conductivity, pH, dissolved oxygen, and temperature	Beginning and end of test in overlying water of one replicate beaker from each treatment.
Dissolved oxygen, temperature	Daily in overlying water of one replicate beaker from each treatment.

Dissolved oxygen is measured using a polarographic oxygen probe calibrated according to the manufacturer's recommendations. The pH is measured using a pH probe and a properly calibrated meter with scale divisions of 0.1 pH units. Temperature is measured with a calibrated mercury thermometer or telethermometer. Conductivity is measured with a conductivity meter. Hardness and alkalinity are measured using titrometric methods. Ammonia-nitrogen is measured using the salicylate colerimetric method (Clin. Chim. Acta 14:403, 1996).

7.8 Growth Measurement: Growth is measured as average dry weight of animals in a test replicate at the end of the test on day 10. Pooled animals from each test replicate are rinsed with deionized water, gently blotted and placed into tared aluminum weigh pans. The pans are dried at 60-90°C to constant weight. The dried organisms are placed into a dessicator and weighed as soon as possible to the nearest 0.01 mg (desirable to use 0.001 mg). The total weight of the dried midge in each pan is divided by the number of midge weighed to obtain an average dry weight per midge.

7.9 Criteria of Test Acceptance: The test results are acceptable if the minimum survival of organisms in the control treatment at the end of the test is at least 70% and the average weight of *C. tentans* in the surviving controls is at least 0.6 mg.

8. DATA ANALYSIS

The endpoints of the toxicity test are survival and growth. Survival is obtained as a direct count of living organisms in each test container at the end of the test. Average midge dry weight, also measured at the end of the test, may be used to compare growth between treatment sediments and the control or reference sediment. Ordinarily the following data analysis is performed. Due to special requirements, alternative methods may be used. The means and standard deviations are calculated for each treatment level. Identification of toxic sediments is established by statistical comparison of test endpoints between test and control or reference sediments. Between treatment comparisons may be made using a Student's t-test or Wilcoxon's Two-Sample test, where each treatment is compared to the control or the reference sediment. An arcsine-square root transformation of proportional data, and tests for normality and heterogeneity of variances, are performed prior to statistical comparisons.

9. REPORTING

The final report of the test results must include all of the following standard information at a minimum: name and identification of the test; the investigator and laboratory; date and time of test beginning and end; information on the test material; information on the source and quality of the overlying/test water; detailed information about the test organisms including acclimation conditions; a description of the experimental design and test chambers and other test conditions including feeding, if any, and water quality; definition of the effect criteria and other observations; responses, if any, in the control treatment; tabulation and statistical analysis of measured responses and a summary table of endpoints; a description of the statistical methods used; any unusual information about the test or deviations from procedures; reference toxicant testing information.

10. STUDY DESIGN ALTERATION

Amendments made to the protocol must be approved by the sponsor and study director and should include a description of the change, the reason for the change, the date the change took effect and the dated signatures of the study director and sponsor. Any deviations in the protocol must be described and recorded in the study raw data.

11. REFERENCE TOXICANT

The reference toxicant test is a standard multi-concentration toxicity test using a specified chemical toxicant to evaluate the performance of test organisms used in the study. Reference toxicant tests are 96-hour, water only exposures, not 10-day sediment exposures. The reference toxicant test is run concurrently. Performance is evaluated by comparing the results of the reference toxicant test with historical results (e.g., control charts) obtained at the laboratory.

12. REFERENCED GUIDELINES

ASTM. 1996. Standard test methods for measuring the toxicity of sediment-associated contaminants with fresh water invertebrates. ASTM Standard Method No. E 1706-95b. Am. Soc. Test. Mat., Philadelphia, PA.

U.S. EPA. 1994. Section 12, Test Method 100.2, Chironomus tentans 10-d Survival and Gowth Test for Sediments, pp. 51-63. In: Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates. EPA/600/R-94/024.

Weber, C.I. (Ed.) 1993. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms (Fourth Edition). EPA/600/4-90/027F.

13. APPROVALS

_____ for _____
Name Date

_____ for Northwestern Aquatic Sciences
Name Date

TOXICITY TEST REPORT

TEST IDENTIFICATION

Test No.: 645-2

Title: Toxicity of freshwater sediments using a 10-day amphipod, *Hyalella azteca*, sediment bioassay.

Protocol No.: NAS-XXX-HA4b, April 7, 1998. Based on ASTM 1996 (Standard test methods for measuring the toxicity of sediment-associated contaminants with fresh water invertebrates, E1706-95b), Am. Soc. Test. Mat. Phila., PA, and EPA Method 100.1 (Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates, EPA/600/R-94/024).

STUDY MANAGEMENT

Study Sponsor: Hart Crowser, Inc., 5 Center Pointe Dr., Suite 240, Lake Oswego, Oregon 97035

Sponsor's Study Monitor: Taku Fuji

Testing Laboratory: Northwestern Aquatic Sciences, P.O. Box 1437, Newport, OR 97365

Test Location: Newport laboratory

Laboratory's Study Personnel: G.J. Irissarri, B.S., Proj. Man./Study Dir.; L.K. Nemeth, M.B.A., QA Officer; R.J. Caldwell, PhD, Senior Toxicologist; G.A. Buhler, B.S., Aq. Toxicologist; G. Hayes, B.S., Tech.

Study Schedule:

Test Beginning: 10-2-01, 1300 hrs.

Test Ending: 10-12-01, 1200 hrs.

Disposition of Study Records: All specimens, raw data, reports and other study records are stored according to Good Laboratory Practice regulations at Northwestern Aquatic Sciences, 3814 Yaquina Bay Rd., Newport, OR 97365.

Good Laboratory Practices: The test was conducted following the principles of Good Laboratory Practices (GLP) as defined in the EPA/TSCA Good Laboratory Practice regulations revised August 17, 1989 (40 CFR Part 792).

Statement of Quality Assurance: The test data were reviewed by the Quality Assurance Unit to assure that the study was performed in accordance with the protocol and standard operating procedures. This report is an accurate reflection of the raw data.

TEST MATERIAL

Test Sediments: Unidentified freshwater test sediments. Details are as follows:

NAS Sample No.	7576F	7577F	7578F
Description	HC-02	HC-08	HC-10
Collection Date	9-17-01	9-17-01	9-17-01
Receipt Date	9-18-01	9-18-01	9-18-01

Control Sediment: The negative control sediment (NAS#7579F) was collected on 9-18-01 from an area approximately one mile east of the Hwy. 101 bridge at Beaver Creek, approx. 8 miles south of Newport, OR. The control sediment was press sieved through a 0.5 mm screen.

Storage: All test and reference sediments were stored at 4°C in the dark until used.

TEST WATER

Source: Moderately hard synthetic water prepared from Milli-Q® deionized water.

Dates of Preparation: 9-28-01, 10-3-01, 10-8-01

Water Quality: pH 8.1, 8.1, 8.0; conductivity 300, 340, 320 µmhos/cm; hardness 85, 94, 94 mg/L as $CaCO_3$; alkalinity 70, 80, 80mg/L as $CaCO_3$.

Pretreatment: Aerated ≥24 hr.

TEST ORGANISMS

Species: *Hyalella azteca*, amphipod.
Age/Size: 10-12 days old
Source: Chesapeake Cultures, Hayes, VA; received 9-28-01
Acclimation: Temperature, 21.8 ± 1.1°C; dissolved oxygen, 9.5 ± 3.1 mg/L; pH, 7.8 ± 0.9; conductivity, 332 ± 22 µmhos/cm; hardness, 145 mg/L as $CaCO_3$; and alkalinity, 135 mg/L as $CaCO_3$.

TEST PROCEDURES AND CONDITIONS

The following is an abbreviated statement of the test procedures and a statement of the test conditions actually employed. See the test protocol (Appendix I) for a more detailed description of the test procedures used in this study.

Test Chambers: 300 ml high-form glass beakers
Test Volumes: 100 ml sediment layer; 175 ml test water.
Replicates/Treatment: 8
Organisms/Treatment: 80
Water Volume Changes: 2 water volumes per day
Aeration: None.
Feeding: Animals are fed 1.5 ml of YCT suspension per beaker daily.
Effects Criteria: Effects Criteria: Survival after 10 days. Death is defined as no visible movement or response to tactile stimulation. Missing organisms were considered to be dead.
Water Quality and Other Test Conditions: The temperature, dissolved oxygen, conductivity, pH, hardness, alkalinity and ammonia-nitrogen were measured in the overlying water of one replicate test container per treatment on days 0 and 10 of the test. Temperature and dissolved oxygen were measured daily in the overlying water of one replicate test container per treatment. Hardness and alkalinity were measured with titrimetric methods. Ammonia-N was measured using Hach test kits based on the salicylate (Clin. Chim. Acta 14:403, 1996) colorimetric method; samples were not distilled prior to analysis. The photoperiod was 16:8, L:D.

DATA ANALYSIS METHODS

Survival was calculated for each replicate as follows:

percent mortality = 100 x (number dead/initial number tested)

Means and standard deviations for the biological endpoints described above, and for water quality data, were computed using Microsoft Excel 2000. The value for mortality for each test sediment was statistically compared against its appropriate reference and control sediment. An arcsine square root transformation was performed on proportional mortality data before analysis. Following determination of normality and homogeneity of variances, a one-tailed Student T-test, Mann-Whitney or Approximate T test was conducted at the 0.05 level of significance. The statistical software used was BioStat (Beta v.2.0c) bioassay software developed by the U.S. Army Corps of Engineers, Seattle District.

PROTOCOL DEVIATIONS

None

REFERENCE TOXICANT TEST

The reference toxicant test is a multi-concentration toxicity test using potassium chloride, to evaluate the performance of the test organisms used in the sediment toxicity test. The performance is evaluated by comparing the results of this test with historical results obtained at the laboratory. A summary of the reference toxicant test result is given below. The reference toxicant test raw data are found in Appendix III.

Test No.: 999-1330
Reference Toxicant and Source: Cadmium as $CdCl_2 \bullet 2½ H_2O$, Mallinckrodt Lot #TNZ, 1.0 mg/ml stock prepared 9-7-00.
Test Date: 10-2-01.
Dilution Water Used: Moderately hard synthetic water prepared from Milli-Q® deionized water.
Result: 96-hr LC50, 11.9 µg/L. This result is within the laboratory's control chart warning limits (3.17 - 18.9 µg/L).

TEST RESULTS

Observations of water quality in the overlying water throughout the test are summarized in Table 1. A detailed tabulation of the water quality results by sample and test day can be found in Appendix II. Interstitial ammonia measurements are listed in Table 2. The means and standard deviations of percent mortality of *Hyalella* exposed for 10 days to sediments are summarized in Table 3. Detailed data organized by sample and replicate, and summary statistics for these observations, are given in Appendix II.

All water quality observations of overlying water temperature and dissolved oxygen were within the protocol specified ranges. Ammonia-N in the overlying water was <0.5 mg/L in all day 0 and day 10 observations. On day 10, overlying water ammonia-N measured <0.5 mg/L except for HC-10 and the control, which contained 0.5 mg/L. Sulfide levels in the overlying water were <0.02 mg/L on days 0 and 10.

The test met acceptability criteria specified in the protocol with 93.8% mean control survival (80% required). The reference toxicant (positive control) result was within the laboratory's control chart limits (11.9 g/L; control chart mean ± 2 S.D. = 11.0 ± 7.87).

None of the sediments tested produced mortality or biomass results that were found to be statistically significantly different from that of the control sediment.

STUDY APPROVAL

_____		_____	
Project Manager/Study Director	Date	Quality Assurance Unit	Date

_____

Laboratory Director Date

Table 1. Summary of water quality conditions during tests of the amphipod, *Hyalella azteca*, exposed to freshwater sediments.

Water Quality Parameter	Mean ± S.D.	Minimum	Maximum	N
Temperature (°C)	22.9 ± 0.2	22.5	23.6	44
Dissolved oxygen (mg/L)	5.8 ± 0.8	4.8	8.2	44
Conductivity (µmhos/cm)	341 ± 37	310	420	8
PH	7.6 ± 0.3	7.4	8.1	8
Hardness (mg/L as $CaCO_3$)	92 ± 4	85	94	8
Alkalinity (mg/L as $CaCO_3$)	80 ± 0	80	80	8
Sulfides (mg/L)	<0.02	<0.02	<0.02	8
Total ammonia (mg/L)	<0.5	<0.5	<0.5	8

Table 2. Interstitial ammonia and pH measurements of test sediments used in test.

Sample description	Ammonia (mg/L)	pH
Control (NAS#7579F)	---	---
HC-02 (NAS#7576F)	<0.05	6.7
HC-08 (NAS#7577F)	1.5	6.9
HC-10 (NAS#7578F)	1.5	6.9

Table 3. Survival of *Hyalella azteca* exposed for 10 days to freshwater sediments. All statistical comparisons were made against the control at the 0.05 level of significance.

Sample description	Percent mortality (Mean ± SD)	Statistically significantly different from the control?
Control (NAS#7579F)	6.3 ± 7.4	---
HC-02 (NAS#7576F)	7.5 ± 14.9	No
HC-08 (NAS#7577F)	6.3 ± 9.2	No
HC-10 (NAS#7578F)	6.3 ± 7.4	No

APPENDIX I

PROTOCOL

TEST PROTOCOL

FRESHWATER AMPHIPOD, *HYALELLA AZTECA*, 10-DAY SEDIMENT TOXICITY TEST

1. INTRODUCTION

1.1 Purpose of Study: The purpose of this study is to characterize the toxicity of freshwater sediments based on survival and, optionally, growth using the amphipod, *Hyalella azteca*.

1.2 Referenced Method: This protocol is based on ASTM Method E 1706-95b (ASTM 1996) and EPA Method 100.1 (EPA/600/R-94/024).

1.3 Summary of Method: A summary of test conditions for the amphipod 10-day sediment toxicity test is tabulated below. The 10-day sediment toxicity test with *Hyalella azteca* is conducted at 23 ± 1°C with a 16L:8D photoperiod at an illuminance of about 50 to 100 footcandles. Test chambers are 300-mL high-form lipless beakers containing 100 mL of sediment and 175 mL of overlying water. Ten 7-14 day old amphipods are used in each replicate. The number of replicates/treatment depends on the objective of the test. Eight replicates are recommended for routine testing. Amphipods in each test chamber are fed 1.5 mL of a YCT food daily. Each chamber receives two volume additions per day of overlying water. Overlying water can be culture water, well water, surface water, site water, or reconstituted water. Test endpoints include survival and/or growth.

1. Test type	whole sediment toxicity test with renewal of overlying water
2. Test duration	10 days
3. Temperature	23 ± 1°C
4. Light quality	daylight flourescent light
5. Illuminance	50-100 footcandles
6. Photoperiod	16L:8D
7. Test chamber size	300-mL high-form lipless beakers, (Pyrex® 1040 or equivalent)
8. Sediment volume	100 mL
9. Overlying water volume	175 mL
10. Renewal overlying water	2 volume additions/day (continuous or intermittent)
11. Age of test organisms	7-14 days old at test initiation
12. Organisms per test chamber	10
13. Replicates per treatment	8 recommended for routine testing (depends on design)
14. Organisms per treatment	80
15. Feeding regime	YCT food, fed 1.5 mL daily/chamber
16. Cleaning	if screens are used, clean as needed
17. Aeration	None, unless DO falls below 40% saturation
18. Overlying (test) water	**Culture water**, well water, surface water, site water or reconstituted water
19. Water quality	Hardness, alkalinity, conductivity, pH, ammonia-N beginning and end; temperature and dissolved oxygen daily
20. Endpoints	Survival (optional, grwoth by dry weight or length)
21. Test acceptability criteria	Minimum control survival of 80%
22. Sample holding	14 days at 4°C in the dark
23. Sample volume required	1L (800 mL per sediment)
24. Reference toxicant	Concurrent testing required

2. STUDY MANAGEMENT

2.1 Sponsor's Name and Address:

_____
_____
_____
_____

2.2 Sponsor's Study Monitor:

_____

2.3 Name of Testing Laboratory:
Northwestern Aquatic Sciences
3814 Yaquina Bay Road, P.O. Box 1437
Newport, OR 97365.

2.4 Test Location: _____

2.5 Laboratory's Personnel to be Assigned to the Study:
Study Director: _____
Quality Assurance Unit: _____
Aquatic Toxicologist: _____
Aquatic Toxicologist: _____

2.6 Proposed Testing Schedule: Tests are to begin within 14 days of sample collection. Reference toxicant test to be run concurrently.

2.7 Good Laboratory Practices: The test is conducted following the principles of Good Laboratory Practices (GLP) as defined in the EPA/TSCA Good Laboratory Practice regulations revised August 17, 1989 (40 CFR Part 792).

3. TEST MATERIAL

The test materials are freshwater sediments. The control, reference, and test sediments are placed in solvent cleaned 1 L glass jars fitted with PTFE-lined screw caps. At the laboratory the samples are stored at 4°C in the dark. The original sealed containers may be stored for up to 14 days prior to testing. If jars are not not full when received or if sediment is removed for testing, headspaces should be filled with nitrogen to retard deterioration. A negative control sediment is collected from a clean site. In addition, a reference sediment, a clean sediment with physical characteristics similar to the test sediments, may be employed as a comparison station.

4. TEST WATER

Test water (overlying water) at NAS is normally moderately hard synthetic water at a hardness of 80-100 mg/L as $CaCO_3$ and alkalinity of 60-70 mg/L as $CaCO_3$. Dilution water is prepared from Milli-Q reagent grade water and reagent grade chemicals. Test water may also be well water, surface water or site water, depending on the study design. Current research is being done by EPA on a new type of reconstituted water that may be used for this test in the future (STM 1996, EPA 1994).

5. TEST ORGANISMS

5.1 Species: amphipod, *Hyalella azteca.*

5.2 Source: Cultured at NAS. Originally purchased from ESE in Gainesville, FL.

5.3 Age: 7-14 days old at start of test; if using growth endpoint, it may be desirable to reduce the age range.

5.4 Acclimation and Pretest Observation: Cultures are maintained at 23 ± 1°C under a 16:8 L:D photoperiod. Cultured amphipods are fed dried maple leaves with occasional Tetramin® flake or rabbit chow supplements. Acclimation of test organisms to the test water may be desirable, depending on culture water, but it is not required by ASTM. If test organisms are to be acclimated, they could be held for 2 hr in a 50:50 mixture of culture water to overlying water, then for 2 hr in a 25:75 mixture, followed by a transfer into 100 % overlying water for 2 hrs. Mortality during the 48-hr prior to testing should not be excessive.

6. DESCRIPTION OF TEST SYSTEM

6.1 Test Chambers and Environmental Control: Test chambers used in the toxicity test are 300-mL high-form lipless glass beakers. Test chambers are maintained at constant temperature by partial immersion in a temperature-controlled water bath or by placement in a temperature-controlled room. Aeration is not empolyed unless dissolved oxygen drops below 40% saturation. The test is conducted under an illuminance of 500 to 1000 lx with a 16L:8D photoperiod.

6.2 Cleaning: All laboratory glassware, including test chambers, is cleaned as described in EPA/600/4-90/027F. New glassware and test systems are soaked 15 minutes in tap water and scrubbed with detergent (or cleaned in automatic dishwasher); rinsed twice with tap water; carefully rinsed once with fresh, dilute (10%, V:V) hydrochloric or nitric acid to remove scale, metals, and bases; rinsed twice with deionized water; rinsed once with acetone to remove organic compounds (using a fume hood or canopy); and rinsed three times with deionized water. Test systems and chambers are rinsed again with dilution water just before use.

7. EXPERIMENTAL DESIGN AND TEST PROCEDURES

7.1 Experimental Design: The test involves exposure of amphipods to test, control, and reference sediments. The sediments are placed on the bottom of the test containers and are overlain with test water. The test exposure is for 10 days. The renewal of overlying water consists of two volume additions per day, either continuous or intermittent. Each treatment consists of eight replicate test containers, each containing 10 organisms. Test chamber positions are completely randomized. Test organisms are randomly distributed to the test chambers. Blind testing is normally used.

7.2 Setup of Test Containers: Sediments are homogenized and placed in test chambers on the day before addition of test organisms. Sediment (100 ml) is placed into each of eight replicate beakers. After addition of the sediment, 175 ml of test water is gently added to each beaker in a manner to prevent resuspension. The overlying water is replaced twice daily. The test begins when amphipods are introduced to the test chambers. Initial water quality measurements are taken prior to the addition of test organisms.

7.3 Effect Criterion: The acute effect criterion used in the amphipod bioassay is mortality, defined as the lack of movement of body or appendages on response to tactile stimulation. The optional chronic effect criterion is growth which is determined by using dry weight measurements, or alternatively body measurements.

7.4 Test Conditions: No aeration is employed unless dissoved oxygen falls below 40% saturation. The test temperature employed is 23 ± 1°C. A 16:8, L:D photoperiod is used. Illumination is supplied by daylight fluorescent lamps at 50-100 footcandles. The overlying water is replaced twice daily.

7.5 Beginning the Test: On the day the test begins, amphipods are impartially counted into small containers of test water (10/container). The test is begun by rinsing test organisms into the equilibrated test containers. If the optional growth endpoint is to be used, time-zero weight/length data should be collected.

7.6 Feeding: Amphipods are fed 1.5 mL of YCT daily per test chamber. A feeding may be skipped if there is a build up of excess food. However, all beakers must be treated similarly.

7.7 Test Duration, Type and Frequency of Observations, and Methods:
The duration of the acute toxicity test is 10 days. The type and frequency of observations to be made are summarized as follows:

TYPE OF OBSERVATION	TIMES OF OBSERVATION (ASTM 1996)
BIOLOGICAL DATA	
Survival, growth	Day 10
PHYSICAL AND CHEMICAL DATA	
Hardness, alkalinity, ammonia-N, conductivity, pH, dissolved oxygen, and temperature	Beginning and end of test in overlying water of one replicate beaker from each treatment.
Dissolved oxygen, temperature	Daily in overlying water of one replicate beaker from each treatment.

Dissolved oxygen is measured using a polarographic oxygen probe calibrated according to the manufacturer's recommendations. The pH is measured using a pH probe and a properly calibrated meter with scale divisions of 0.1 pH units. Temperature is measured with a calibrated mercury thermometer or telethermometer. Conductivity is measured with a conductivity meter. Hardness and alkalinity are measured using titrometric methods. Ammonia-nitrogen is measured using the salicylate colerimetric method (Clin. Chim. Acta 14:403, 1996).

Overlying water should be sampled just before water renewal from about 1 to 2 cm above the sediment surface using a pipet. It may be necessay to pool water samples from individual replicates. The pipet should be checked to make sure no organisms are removed during sampling of overlying water.

7.8 Growth Measurement: Growth is measured as average dry weight of animals in a test replicate at the end of the test on day 10 or average total length of preserved animals. Pooled animals from each test replicate are rinsed with deionized water, gently blotted and placed into tared aluminum weigh pans. The pans are dried at 60-90°C to constant weight. The dried amphipods are placed into a dessicator and weighed as soon as possible to the nearest 0.01 mg (desirable to use 0.001 mg). The total weight of the dried amphipods in each pan is divided by the number of amphipods weighed to obtain an average dry weight per surviving amphipod per replicate.

If the length growth endpoint is to be used, either in place of or along with the weight endpoint, amphipods from each replicate are pooled and preserved in vials. Later, the preserved amphipods from one vial are carefully stretched out on a glass microscope slide and quickly measured using a dissecting microscope fitted with a calibrated ocular micrometer. Then, if weight is to be determined, the animals are quickly transferred to tared aluminum foil cups. Fine-tipped forceps are required for these handling operations.

If both measurements are to be used, total length measurements are taken first, then the animals are transferred to tared aluminum foil cups for weighing.

7.9 Criteria of Test Acceptance: The test results are acceptable if the minimum survival of organisms in the control treatment at the end of the test is at least 80%.

8. DATA ANALYSIS

The endpoints of the toxicity test are survival and growth (optional). Survival is obtained as a direct count of living organisms in each test container at the end of the test. Average amphipod dry weight, also measured at the end of the test, may be used to compare growth between treatment sediments and the control or reference sediment. Ordinarily the following data analysis is performed. Due to special requirements, alternative methods may be used. The means and standard deviations are calculated for each

treatment level. Identification of toxic sediments is established by statistical comparison of test endpoints between test and control or reference sediments. Between treatment comparisons may be made using a Student's t-test or Wilcoxon's Two-Sample test, where each treatment is compared to the control or the reference sediment. An arcsine-square root transformation of proportional data, and tests for normality and heterogeneity of variances, are performed prior to statistical comparisons.

9. REPORTING

The final report of the test results must include all of the following standard information at a minimum: name and identification of the test; the investigator and laboratory; date and time of test beginning and end; information on the test material; information on the source and quality of the overlying/test water; detailed information about the test organisms including acclimation conditions; a description of the experimental design and test chambers and other test conditions including feeding, if any, and water quality; definition of the effect criteria and other observations; responses, if any, in the control treatment; tabulation and statistical analysis of measured responses and a summary table of endpoints; a description of the statistical methods used; any unusual information about the test or deviations from procedures; reference toxicant testing information.

10. STUDY DESIGN ALTERATION

Amendments made to the protocol must be approved by the sponsor and study director and should include a description of the change, the reason for the change, the date the change took effect and the dated signatures of the study director and sponsor. Any deviations in the protocol must be described and recorded in the study raw data.

11. REFERENCE TOXICANT

The reference toxicant test is a standard multi-concentration toxicity test using a specified chemical toxicant to evaluate the performance of test organisms used in the study. Reference toxicant tests are 96-hour, water only exposures, not 10-day sediment exposures. The reference toxicant test is run concurrently. Performance is evaluated by comparing the results of the reference toxicant test with historical results (e.g., control charts) obtained at the laboratory.

12. REFERENCED GUIDELINES

ASTM. 1996. Standard test methods for measuring the toxicity of sediment-associated contaminants with fresh water invertebrates. ASTM Standard Method No. E 1706-95b. Am. Soc. Test. Mat., Philadelphia, PA.

U.S. EPA. 1994. Section 11, Test Method 100.1, Hyalella azteca 10-d Survival Test for Sediments, pp. 44-50 In: Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates. EPA/600/R-94/024.

Weber, C.I. (Ed.) 1993. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms (Fourth Edition). EPA/600/4-90/027F.

13. APPROVALS

_____ for _____
Name Date

_____ for Northwestern Aquatic Sciences
Name Date

TOXICITY TEST REPORT

TEST IDENTIFICATION

Test No.: 645-3

Title: *Lumbriculus variegatus* 28-day bioaccumulation exposure to freshwater sediments.

Protocol No.: NAS-XXX-LV5 (Revision 2), August 20, 2001. Based on EPA Method 100.3 (Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates, EPA/600/R-94/024) and the "Inland Testing Manual" (EPA-B-98-004).

STUDY MANAGEMENT

Study Sponsor: Hart Crowser, Inc., 5 Center Pointe Dr., Suite 240, Lake Oswego, Oregon 97035

Sponsor's Study Monitor: Taku Fuji

Testing Laboratory: Northwestern Aquatic Sciences, P.O. Box 1437, Newport, OR 97365

Test Location: Newport laboratory

Laboratory's Study Personnel: G.J. Irissarri, B.S., Proj. Man./Study Dir.; L.K. Nemeth, M.B.A., QA Officer; R.J. Caldwell, PhD, Senior Toxicologist; G.A. Buhler, B.S., Aq. Toxicologist; M. S. Redmond, M.S., Aq. Toxicologist; G. Hayes, B.S., Tech.

Study Schedule:

Test Beginning: 10-2-01, 1610

Test Ending: 10-30-01, 1500

Disposition of Study Records: All specimens, raw data, reports and other study records are stored according to Good Laboratory Practice regulations at Northwestern Aquatic Sciences, 3814 Yaquina Bay Rd., Newport, OR 97365.

Good Laboratory Practices: The test was conducted following the principles of Good Laboratory Practices (GLP) as defined in the EPA/TSCA Good Laboratory Practice regulations revised August 17, 1989 (40 CFR Part 792).

Statement of Quality Assurance: The test data were reviewed by the Quality Assurance Unit to assure that the study was performed in accordance with the protocol and standard operating procedures. This report is an accurate reflection of the raw data.

TEST MATERIAL

Test Sediments: Unidentified freshwater test sediments. Details are as follows:

NAS Sample No.	7577F	7578F
Description	HC-08	HC-10
Collection Date	9-17-01	9-17-01
Receipt Date	9-18-01	9-18-01

Control Sediment: The negative control sediment (NAS#7579F) was collected on 9-18-01 from an area approximately one mile east of the Hwy. 101 bridge at Beaver Creek, approx. 8 miles south of Newport, OR. The control sediment was press sieved through a 0.5 mm screen.

Storage: All test and reference sediments were stored at 4°C in the dark until used.

.

TEST WATER

Source: Moderately hard synthetic water prepared from Milli-Q® deionized water.

Dates of Preparation: 9-28-01, 10-3-01, 10-8-01, 10-12-01, 10-17-01, 10-23-01

Water Quality: pH 8.1, 8.1, 8.0, 8.0, 8.3, 8.3; conductivity 280, 340, 320, 300, 320, 330 µmhos/cm; hardness 102, 94, 94, 85, 85, 102 mg/L as $CaCO_3$; alkalinity 80, 80, 80, 70, 80,80 mg/L as $CaCO_3$.

Pretreatment: Aerated ≥24 hr.

TEST ORGANISMS

Species: *Lumbriculus variegatus.*
Age: Adult
Source: Environmental Consulting & Testing, Superior, WI. Received 9-25-01.
Acclimation: Worms were placed in small aquaria with moderately hard water and aeration. Animals were fed TetraFin suspension during holding. Water quality conditions for the week prior to testing averaged: temperature, 22.7 ± 1.8°C; dissolved oxygen, 7.0 ± 3.3 mg/L; pH, 7.6 ± 0.6; conductivity 321 ± 29 umhos/cm; hardness, 85 ± 29 mg/L as CaCO3 and alkalinity, 97 ± 6 mg/L as CaCO3.

TEST PROCEDURES AND CONDITIONS

Test Design and Summary of Test Procedures: The bioaccumulation test required *L. variegatus* to be exposed for 28 days to test and reference sediments. Four liters of each sediment were placed in the bottom of five-gallon aquaria and filled with 8 liters of moderately hard water one day prior to the date that the worm exposure was to begin. Test chambers were placed in a temperature-controlled room. On the day of test initiation, approximately 12 grams of worms were placed into each test chamber. Five replicates of 0-time samples were collected and sent to Columbia Analytical Services for analysis. Three times per week, the overlying water was siphoned out and changed. Five replicate aquaria were employed for each sediment treatment, which provided five replicates of tissue for chemical analysis. The exposure temperatures were 23 ± 1°C. During the exposure period, test chambers were examined daily for sediment avoidance behavior. After 28 days, worms were removed from the test sediments, cleaned and returned to clean water-filled beakers without sediment to depurate for 6-8 hours. Worms were then removed from the depuration chambers, rinsed with Milli-Q® deionized water, lightly blotted, weighed and put into separate jars and frozen. Animals were shipped to the analytical laboratory (Columbia Analytical Services) for analysis.

Test Chambers: 5 gallon glass aquaria (8" x 16" x 10").
Test Volumes: 4.0 L sediment and approximately 8 L of overlying seawater
Replicates/Treatment: 5
Organisms/Treatment: approximately 60 grams (~ 12 grams per tank)
Water Volume Changes: 75% of overlying water replaced three times per week.
Aeration: Provided using 1 ml glass pipet placed 3-5 cm above the sediment surface; aeration rate of approximately 1 bubble per second.
Feeding: None
Effects Criteria: The primary purpose of the bioaccumulation study was to measure the concentrations of selected sediment contaminants in the tissues of the worms after 28 days of exposure, rather than to observe organism physiological or behavioral responses as in ordinary toxicology tests. NAS performed the laboratory exposure only. After a 6-8-hr depuration period, the surviving worms were frozen and shipped under chain-of-custody to the analytical laboratory (Columbia Analytical Services).

Water Quality and Other Test Conditions: The temperature, dissolved oxygen, pH, conductivity, hardness, and alkalinity were measured in one replicate of each treatment on Days 0, 8, 14, 21, 28 and during the depuration period. Ammonia-N was also measured in one replicate of each treatment on Days 0, 3, 5, 8, 14, 21 and 28. Temperature and dissolved oxygen were measured in one replicate daily during the 28-day exposure. The values of individual water quality measurements are to be found in the raw data (Appendix II). The overall means, standard deviations, and the minimum and maximum values for the water quality parameters taken during the 28-day exposure are given in table 1 below.

DATA ANALYSIS METHODS

Means and standard deviations for water quality parameters were calculated using Microsoft Excel 5.0.

PROTOCOL DEVIATIONS

None

REFERENCE TOXICANT TEST

Test No.: 999-1331
Reference Toxicant and Source: Potassium chloride (Mallinckrodt, Lot No. 6845 KXAT).
Test Date: 10-2-01
Dilution Water Used: Moderately hard synthetic water.
Result: LC50, 0.55 µg/L. This result is within the laboratory's control chart warning limits (0.48 – 0.79µg/L).

TEST RESULTS

Interstitial ammonia levels for HC-08 (NAS#7577F) and HC-10 (NAS#7578F) prior to test initiation was 1.5 mg/L for both sediments (see Table 2). Water quality measurements and animal biomass after the 28-day exposure period are given in Table 1 and Table 3.

STUDY APPROVAL

Project Manager/Study Director	Date	Quality Assurance Unit	Date

Manager, Toxicology Date

Table 1. Summary of Water Quality Measurements of 28-Day Bioaccumulation Exposure to Test Sediments.

	Mean	S.D.	Min.	Max.	n
Temperature (°C)	22.6	0.2	22.1	23.2	90
Dissolved oxygen (mg/L)	7.0	0.6	5.4	8.3	90
Conductivity (umhos/cm)	339	57	230	480	18
pH	7.6	0.2	7.3	7.9	18
Hardness (as mg/L $CaCO_3$)	86	2	85	94	15
Alkalinity(as mg/L $CaCO_3$)	75	6	60	80	15
Sulfide (mg/L)	<0.02	---	<0.02	<0.02	15
Ammonia-N (mg/L)	---	---	<0.5	1.0	15

Table 2. Interstitial ammonia and pH prior to test initiation.

Sediment Description	Ammonia (mg/L)	pH
HC-08 (NAS#7577F)	1.5	6.9
HC-10 (NAS#7578F)	1.5	6.9

Table 3. Biomass of Worms, *Lumbriculus variegatus*, Exposed for 28 Days to Test Sediments.

Sample Description	Initial Biomass (grams)					Final Biomass (grams)				
	1	2	3	4	5	1	2	3	4	5
HC-08 (NAS#7577F)	12.5	12.6	12.5	12.2	12.6	10.0	7.6	8.9	8.7	10.6
HC-10 (NAS#7578F)	12.5	12.5	12.3	12.6	12.2	10.3	7.9	9.3	9.1	9.3
Control (7579F)	12.4	12.5	12.1	12.7	12.3	9.8	7.3	9.2	10.2	9.3

APPENDIX I

PROTOCOL

TEST PROTOCOL

FRESHWATER OLIGOCHAETE, *LUMBRICULUS VARIEGATUS*, 28-DAY SEDIMENT BIOACCUMULATION

1. INTRODUCTION

1.1 Purpose of Study: Laboratory sediment bioaccumulation tests provide an estimate of contaminant uptake by benthic infauna. The purpose of this study is to expose oligochaetes (*Lumbriculus variegatus*) to freshwater sediments for 28 days so that they may bioaccumulate sediment-associated contaminants. After the bioaccumulation period, worms are frozen for subsequent tissue analysis.

1.2 Referenced Method: This protocol is based on, and complies in all essential respects, with EPA Method 100.3 (EPA/600/R-99/064). An optional schedule of overlying water renewal based on the "Inland Testing Manual" (EPA-B-98-004) has been included for increased test flexibility. Some modifications of scale have also been incorporated to increase test flexibililty.

1.3 Summary of Method: A summary of test conditions for the 28-day oligochaete bioaccumulation test is tabulated on page 6.

Because *L. variegatus* is quite sensitive to some contaminants, a 96-hour toxicity screening test is performed to ensure that the samples are not overly toxic prior to setting up the bioacummulation test. The screening test is conducted in 300 ml test chambers containing 100 ml of sediment and 175 ml of overlying water. The test is conducted at 23°C, 16:8 photoperiod, with twice daily renewal of overlying water. The screening test is inititated with ten adult oligochaetes per replicate and a minimum of four replicates per treatment. Animals are not fed during the test. If there is significant mortality or animals are exhibiting avoidance behavior, then bioaccumulation testing with *L. variegatus* may not be possible or appropriate. If the screening test indicates that the test sediments are not toxic and animals are not avoiding the sediment, then the bioaccumulation test is started.

The 28-day bioaccumulation test with *Lumbriculus variegatus* is conducted at 23°C with a 16L:8D photoperiod at an illuminance of about 50-100 ft-c. Test chambers are typically 4-8 L (1-2 gal) aquaria containing 1-2 L of sediment (uniform amount across all containers) and 2-4 L of overlying water. The test is stocked at densities of 2-5 g wet biomass per replicate test chamber depending upon chemistry requirements as long as the minimum ratio of sediment TOC to organism dry weight of 50:1 is not violated. Larger test systems (e.g. 5 gal aquaria holding up to 4 L of sediment, 8 L of water, and stocked with 10-12 g of worms) may be employed if greater tissue volumes are required. The takedown of these larger systems may, however, become prohibitively labor intensive (> 30 man-hours per sample or control treatments). Sediments are added to test chambers and overlying water exchanged twice during the 24 hours prior to test initiation. Adult oligochaetes are used. There are five replicates per treatment. Test organisms are not fed during the test. Gentle aeration is employed to insure that dissolved oxygen concentration does not fall below 2.5 mg/L. Each chamber receives two volume additions per day of overlying water (optionally three times per week, or less, as dictated by study needs). Overlying water can be culture water, well water, surface water, site water, reconstituted water, or dechlorinated municipal water. The test endpoint is bioaccumulation.

2. STUDY MANAGEMENT

2.1 Sponsor's Name and Address:

_____
_____
_____
_____

2.2 Sponsor's Study Monitor:

_____

2.3 Name of Testing Laboratory:
Northwestern Aquatic Sciences
3814 Yaquina Bay Road, P.O. Box 1437
Newport, OR 97365.

2.4 Test Location: _____

2.5 Laboratory's Personnel to be Assigned to the Study:
Study Director: _____
Quality Assurance Unit: _____
Aquatic Toxicologist: _____
Aquatic Toxicologist: _____

2.6 Proposed Testing Schedule: The time between sediment collection and use in testing should be kept to a minimum; therefore, bioaccumulation testing is started as soon after sample receipt as logistically possible. For many applications a maximum holding time of 8 weeks is employed.

2.7 Good Laboratory Practices: The test is conducted following the principles of Good Laboratory Practices (GLP) as defined in the EPA/TSCA Good Laboratory Practice regulations revised August 17, 1989 (40 CFR Part 792).

3. TEST MATERIAL

The test materials are freshwater sediments. It is recommended that he control, reference, and test sediments be placed in clean, air-tight containers. Sediments for metals bioaccumulation should be stored in the absence of air to minimize the oxidation of reduced forms. Nitrogen can be used to fill the headspace in the containers. Glass containers are recommended for sediments polluted with either metals or organics, although high-density polyethylene and PTFE containers are also acceptable. Large organisms and extraneous materials, such as bivalves or twigs, should be removed from the sediments before storing. At the laboratory the samples are stored at 4°C in the dark. The time between sediment collection and use in testing should be kept to a minimum. A maximum holding time of eight weeks is recommended. A negative control sediment is collected from a clean site or the animal collection site, and should contain no or very low concentrations of the contaminant(s) of concern. In addition, a reference sediment is normally employed as a comparison station when evaluating dredged materials

4. TEST WATER

Test water (overlying water) at NAS is normally moderately hard synthetic water at a hardness of 80-100 mg/L as $CaCO_3$ and alkalinity of 60-70 mg/L as $CaCO_3$. Dilution water is prepared from dechlorinated and deionized municipal water or Milli-Q reagent grade water and reagent grade chemicals. Test water may also be well water, surface water, or site water depending on the study needs.

5. TEST ORGANISMS

5.1 Species: oligochaete, *Lumbriculus variegatus.*

5.2. Source: Commercial suppliers or laboratory cultures

5.3 Age: Adult

5.4 Acclimation and Pretest Observation: After receipt, worms should be held in the laboratory for at least 24 hours prior to test initiation in order to assess their health and acclimate them to test conditions. Mortality during the holding period should not be excessive.

6. DESCRIPTION OF TEST SYSTEM

6.1 Test Chambers and Environmental Control: Test chambers generally used in the toxicity test are 4-8 L (1 – 2 gal) aquaria. Test chambers are maintained at constant temperature by partial immersion in a temperature-controlled water bath or by placement in a temperature-controlled room. Gentle aeration is employed to insure that dissolved oxygen concentration does not fall below 2.5 mg/L. The test is conducted under an illuminance of 10-20 $\mu E/m^2/s$ (50-100 ft-c) with a 16L:8D photoperiod.

6.2 Cleaning: All laboratory glassware, including test chambers, is cleaned as described in EPA/600/4-90/027F. New glassware and test systems are soaked 15 minutes in tap water and scrubbed with detergent (or cleaned in automatic dishwasher); rinsed twice with tap water; carefully rinsed once with fresh, dilute (10%, V:V) hydrochloric or nitric acid to remove scale, metals, and bases; rinsed twice with deionized water; rinsed once with acetone to remove organic compounds (using a fume hood or canopy); and rinsed three times with deionized water. Test systems and chambers are rinsed again with dilution water just before use.

7. EXPERIMENTAL DESIGN AND TEST PROCEDURES

7.1 Experimental Design: The test involves exposure of worms to test, control, and reference sediments. The sediments are placed on the bottom of the test containers and are overlain with test water. The test exposure is for 28 days. Static replacement of overlying water is performed twice daily at approximately 12 hour intervals (EPA/600/R-99/064). Optionally, overlying water may be renewed three times per week, or less, as dictated by study needs (EPA 823-B-98-004). Each treatment consists of five replicate test containers, each containing enough organisms to provide approximately 2- to 5-g wet weight. The animals are added to each replicate at about 1.33 times the target stocking weight (the additional 33% accounts for the excess weight from water in the nonblotted oligochaetes). Test chamber positions are completely randomized. Test organisms are randomly distributed to the test chambers. Animals are placed on the sediment surface and allowed to bury.

7.2 Setup of Test Containers: Sediments are homogenized and placed in test chambers on the day before addition of test organisms. From 1-2 L of sediment is placed into each of five replicate aquaria. After addition of the sediment, 2-4 L of test water is gently added to each test container in a manner to prevent resuspension. The overlying water is replaced twice daily, starting on Day -1 (twice daily renewal schedule only). The test begins when worms are introduced to the test chambers. Initial water quality measurements are taken prior to the addition of test organisms.

7.3 Test Conditions: Gentle aeration is employed to insure that dissolved oxygen concentration does not fall below 2.5 mg/L. The test temperature employed is 23 ± 1°C). A 16:8, L:D photoperiod is used. Illumination is supplied by daylight fluorescent lamps at 10-20 $\mu E/m^2/s$ (50-100 ft-c).

7.4 Beginning the Test: The test is begun by adding the organisms to the equilibrated test containers as previously described. A five replicate zero-time sample of test animals is preserved (frozen) for analysis of initial concentrations of chemicals of concern. Mean group weights should be measured on a subset of at least 100 organisms used to start the test.

7.5 Feeding: None.

7.6 Test Duration, Type and Frequency of Observations, and Methods: The duration of the bioaccumulation test is 28 days. The type and frequency of observations to be made are summarized as follows:

TYPE OF OBSERVATION	TIMES OF OBSERVATION
BIOLOGICAL DATA	
Observations on behavior	Daily
PHYSICAL AND CHEMICAL DATA	
Hardness, alkalinity, conductivity, pH and total ammonia	Beginning and end of test in overlying water. One replicate per treatment. (optionally on days 0, 7, 14, 21, 28).
Dissolved oxygen, temperature	Daily in overlying water. One replicate per treatment

Dissolved oxygen is measured using a polarographic oxygen probe calibrated according to the manufacturer's recommendations. The pH is measured using a pH probe and a properly calibrated meter with scale divisions of 0.1 pH units. Temperature is measured with a calibrated mercury thermometer or telethermometer. Conductivity is measured with a conductivity meter. Hardness and alkalinity are measured using titrometric methods. Ammonia-nitrogen is measured using the salicylate colerimetric method (Clin. Chim. Acta 14:403, 1996).

7.7 Test Termination and Depuration: At test termination, animals are removed from the sediment by gently sieving test chamber contents through a fine-mesh sieve sufficiently small to retain the oligochaetes (e.g., 500 μm mesh). Immobile organisms should be considered dead. Live oligochaetes from an individual replicate should be transferred to a 1-L beaker containing overlying water without sediment for 6 to 8 hours (or up to 24 hours if needed, depending on the chemicals of concern) to depurate, or eliminate gut contents. Aeration may be required if dissolved oxygen falls below 2.5 mg/L. Each sample is then weighed, placed in a clean container, and frozen for later tissue residue analysis. If an estimate of dry weight is needed, a subsample should be dried to a constant weight at about 60 to 90C, then brought to room temperature in a dessicator and weighed to the nearest 0.01 mg. Ash-free weight may be desirable in some instances.

7.8 Criteria of Test Acceptance: The test results are acceptable if the test organisms burrow into the sediments; the test should be considered invalid if overt sediment avoidance is observed.

8. DATA ANALYSIS

The endpoint of the test is bioaccumulation. Surviving worms are depurated for 6 to 8 hours and then frozen for subsequent tissue analysis. Data analysis consists of calculating means and standard deviations for tissue chemical concentrations and water quality parameters. Statistical comparisons of treatment groups may be done using standard hypothesis test procedures (i.e. test for normality and homogeneity followed by parametric or non-parmetric comparison tests as appropriate).

9. REPORTING

The final report of the test results must include all of the following standard information at a minimum (except that tabulation and analysis of tissue chemical concentrations is optional depending on client

requirements): name and identification of the test; the investigator and laboratory; date and time of test beginning and end; information on the test material; information on the source and quality of the overlying/test water; detailed information about the test organisms including acclimation conditions; a description of the experimental design and test chambers and other test conditions including water quality; definition of the effect criteria and other observations; responses, if any, in the control treatment, tabulation and statistical analysis of measured responses and a summary table of endpoints; a description of the statistical methods used; any unusual information about the test or deviations from procedures; results of the initial screening toxicity test.

10. STUDY DESIGN ALTERATION

Amendments made to the protocol must be approved by the sponsor and study director and should include a description of the change, the reason for the change, the date the change took effect and the dated signatures of the study director and sponsor. Any deviations in the protocol must be described and recorded in the study raw data.

11. REFERENCED AND/OR CONSULTED GUIDELINES

ASTM. 1997. Determination of the Bioaccumulation of Sediment-Associated Contaminants by Benthic Invertebrates. ASTM Standard Method No. E 1688 – 97a. Am. Soc. Test. Mat., Philadelphia, PA.

Portland Harbor Sediment Management Plan. June 25, 1999. Oregon Department of Environmental Qualtiy.

U.S. EPA. 2000. Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates. Second Edition. EPA/600/R-99/064.

U.S. EPA. 1998. Evaluation of Dredged Material Proposed for Discharge in Waters of the U.S. – Testing Manual: Inland Testing Manual. EPA 823-B-98-004.

Weber, C.I. (Ed.) 1993. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms (Fourth Edition). EPA/600/4-90/027F.

12. APPROVALS

_____ for _____
Name Date

_____ for Northwestern Aquatic Sciences
Name Date

SUMMARY OF TEST CONDITIONS

***LUMBRICULUS VARIEGATUS*,**
28-DAY SEDIMENT BIOACCUMULATION

1. Test type:	Static Renewal
2. Test duration:	28 days
3. Temperature:	23 ± 1° C
4. Light quality:	Ambient Laboratory
5. Light itensity:	10-20 μE/m^2/s (50-100 ft-c)
6. Photoperiod:	16L:8D
7. Test chamber size:	1 - 2 gal aquaria (optional-5 gal)
8. Sediment volume:	1 - 2 L (optional-5 L)
9. Overlying water volume:	2 - 4 L (optional-10 L)
10. Renewal of overlying water:	Twice daily; approx. 12-hr intervals 3x/wk or more (optional)
11. Age of test organisms:	Adults
12. Loading of organisms in chamber:	2 – 5 g/replicate (optional 10 – 12 g/replicate). Ratio of sediment TOC to organism dry weight ≥ 50:1
13. Replicates per sediment:	5
14. Test chamber cleaning:	None
15. Feeding:	None
16. Aeration:	Gentle, as needed
17. Overlying water:	Culture water, well water, surface water, site water, or reconstituted water, or dechlorinated municipal water.
18. Study treatments:	Site sediment(s), a reference sediment, and a control sediment.
19. Overlying water quality:	Hardness, alkalinity, conductivity, pH, and ammonia at the beginning and end of a test. Temperature and dissolved oxygen daily.
20. Endpoint:	Bioaccumulation
21. Sample holding requirements:	< 8 weeks
22. Sample volume required:	5 – 10 L (optional-25 L)
23. Test acceptability:	1. Adequate mass of test organisms at test completion for detection of target analytes. 2. Test organisms must burrow into the test sediments.

TOXICITY TEST REPORT

TEST IDENTIFICATION

Test No.: 645-4
Title: *Corbicula fluminea* 28-day bioaccumulation exposure to freshwater sediments.
Protocol No.: NAS-XXX-CF5, August 20, 2001. Based on 1) Portland Harbor Sediment Management Plan - Appendix G; 2) EPA/600/R-93/183; 3) EPA 823-B-98-004; 4) and ASTM E-1688-97a.

STUDY MANAGEMENT

Study Sponsor: Hart Crowser, Inc., 5 Center Pointe Dr., Suite 240, Lake Oswego, Oregon 97035
Sponsor's Study Monitor: Taku Fuji
Testing Laboratory: Northwestern Aquatic Sciences, P.O. Box 1437, Newport, OR 97365
Test Location: Newport laboratory
Laboratory's Study Personnel: G.J. Irissarri, B.S., Proj. Man./Study Dir.; L.K. Nemeth, M.B.A., QA Officer; R.J. Caldwell, PhD, Senior Toxicologist; G.A. Buhler, B.S., Aq. Toxicologist; G. Hayes, B.S., Tech.
Study Schedule:
Test Beginning: 10-29-01, 1130
Test Ending: 11-26-01, 1500
Disposition of Study Records: All specimens, raw data, reports and other study records are stored according to Good Laboratory Practice regulations at Northwestern Aquatic Sciences, 3814 Yaquina Bay Rd., Newport, OR 97365.
Good Laboratory Practices: The test was conducted following the principles of Good Laboratory Practices (GLP) as defined in the EPA/TSCA Good Laboratory Practice regulations revised August 17, 1989 (40 CFR Part 792).
Statement of Quality Assurance: The test data were reviewed by the Quality Assurance Unit to assure that the study was performed in accordance with the protocol and standard operating procedures. This report is an accurate reflection of the raw data.

TEST MATERIAL

Test Sediments: Unidentified freshwater test sediments. Details are as follows:

NAS Sample No.	7577F	7578F
Description	HC-08	HC-10
Collection Date	9-17-01	9-17-01
Receipt Date	9-18-01	9-18-01

Control Sediment: The negative control sediment (NAS#7579F) was collected on 9-18-01 from an area approximately one mile east of the Hwy. 101 bridge at Beaver Creek, approx. 8 miles south of Newport, OR. The control sediment was press sieved through a 0.5 mm screen.
Storage: All test and reference sediments were stored at 4°C in the dark until used.
.

TEST WATER

Source: Moderately hard synthetic water prepared from Milli-Q® deionized water.
Dates of Preparation: 10-25-01, 10-29-01, 11-5-01, 11-9-01, 11-12-01, 11-16-01, 11-19-01
Water Quality: pH 8.0, 8.1, 8.4, 8.1, 8.2, 8.0, 8.1; conductivity 330, 320, 330, 320, 300, 270, 310 µmhos/cm; hardness 94, 94, 94, 94, 85, 94, 94 mg/L as $CaCO_3$; alkalinity 80, 80, 70, 80, 70, 70,80 mg/L as $CaCO_3$.
Pretreatment: Aerated ≥24 hr.

TEST ORGANISMS

Species: *Corbicula fluminea*.
Age: Adult
Source: T.A.I. Environmental Sciences, Mobile, AL. Received 10-22-01.
Acclimation: Clams were placed in small aquaria with moderately hard water and aeration. Animals were not fed during holding. Water quality conditions for the week prior to testing averaged: temperature, 19.3 ± 2.6°C; dissolved oxygen, 10.1 ± 2.4 mg/L; pH, 7.8 ± 0.4; conductivity 409 ± 40 umhos/cm; hardness, 159 ± 39 mg/L as CaCO3 and alkalinity, 133 ± 32 mg/L as CaCO3.

TEST PROCEDURES AND CONDITIONS

Test Design and Summary of Test Procedures: The bioaccumulation test required *C. fluminea* to be exposed for 28 days to test and reference sediments. Seven liters of each sediment were placed in the bottom of ten-gallon aquaria and filled with 18 liters of moderately hard water one day prior to the date that the clam exposure was to begin. Test chambers were placed in a temperature-controlled room. On the day of test initiation, 43 clams (approximately 35 grams of wet tissue weight) were placed into each test chamber. Five replicates of 0-time samples were collected and sent to Columbia Analytical Services for analysis. Three times per week, the overlying water was siphoned out and changed. Five replicate aquaria were employed for each sediment treatment, which provided five replicates of tissue for chemical analysis. The exposure temperatures were 20 ± 1°C. During the exposure period, test chambers were examined daily for sediment avoidance behavior. After 28 days, clams were removed from the test sediments, cleaned and returned to clean water-filled aquaria without sediment to depurate for 24 hours. Clams were then removed from the depuration chambers, rinsed with Milli-Q® deionized water, lightly blotted, and put into separate jars and frozen. Animals were shipped to the analytical laboratory (Columbia Analytical Services) for analysis.

Test Chambers: 10 gallon glass aquaria (10" x 20" x 12").
Test Volumes: 7.0 L sediment and approximately 18 L of overlying seawater
Replicates/Treatment: 5
Organisms/Treatment: 215 clams or approximately 175 grams wet tissue weight (~ 35 grams per tank)
Water Volume Changes: 75% of overlying water replaced three times per week.
Aeration: Provided using 1 ml glass pipet placed 3-5 cm above the sediment surface.
Feeding: None
Effects Criteria: The primary purpose of the bioaccumulation study was to measure the concentrations of selected sediment contaminants in the tissues of the clams after 28 days of exposure, rather than to observe organism physiological or behavioral responses as in ordinary toxicology tests. NAS performed the laboratory exposure only. After a 24-hr depuration period, the surviving clams were frozen and shipped under chain-of-custody to the analytical laboratory (Columbia Analytical Services).

Water Quality and Other Test Conditions: The temperature, dissolved oxygen, pH, conductivity, hardness, and alkalinity were measured in one replicate of each treatment on Days 0, 7, 14, 21, 28 and the depuration period. Ammonia-N and sulfides were also measured in one replicate of each treatment on Days 0, 7, 14, 21 and 28. Temperature and dissolved oxygen were measured in one replicate daily during the 28-day exposure. The values of individual water quality measurements are to be found in the raw data (Appendix II). The overall means, standard deviations, and the minimum and maximum values for the water quality parameters taken during the 28-day exposure and depuration period are given in table 1 below.

DATA ANALYSIS METHODS

Means and standard deviations for water quality parameters were calculated using Microsoft Excel 5.0.

PROTOCOL DEVIATIONS

None

TEST RESULTS

Interstitial ammonia levels for HC-08 (NAS#7577F), HC-10 (NAS#7578F) and the control sediment (NAS#7579F) prior to test initiation was <2.5 mg/L for all sediments (see Table 2). Water quality measurements for the 28-day exposure and depuration period are summarized in Table 1. Average clam size and weights at test initiation are listed in Table 3. Table 4 lists clam survival at end of the 28-day exposure period.

STUDY APPROVAL

_____		_____	
Project Manager/Study Director	Date	Quality Assurance Unit	Date

_____

Manager, Toxicology Date

Table 1. Summary of Water Quality Measurements of 28-Day Bioaccumulation Exposure to Test Sediments.

	Mean	S.D.	Min.	Max.	n
Temperature (°C)	20.1	0.4	19.4	20.9	90
Dissolved oxygen (mg/L)	8.5	0.4	7.0	9.0	90
Conductivity (umhos/cm)	352	50	310	510	18
pH	7.7	0.2	7.3	8.2	18
Hardness (as mg/L $CaCO_3$)	93	5	85	102	18
Alkalinity(as mg/L $CaCO_3$)	84	6	80	100	18
Sulfide (mg/L)	<0.02	---	<0.02	<0.02	15
Ammonia-N (mg/L)	---	---	<0.5	<0.5	15

Table 2. Interstitial ammonia and pH prior to test initiation.

Sediment Description	Ammonia (mg/L)	pH
HC-08 (NAS#7577F)	<2.5	6.6
HC-10 (NAS#7578F)	<2.5	6.7
Control (NAS#7579F)	<2.5	6.9

Table 3. Shell Width, Whole Clam Weight, and Wet Tissue Weight of *Corbicula Fluminea* used to Calculate Number of Clams to Add to Sediment Exposure Tanks.

	Shell Width (cm)	Whole Clam Weight (gm)	Wet Weight of Clam Tissue (gm)
	2.6	7.06	1.11
	2.5	5.79	0.83
	2.3	5.01	0.64
	2.6	7.08	0.83
	2.6	6.34	0.87
	2.5	5.99	0.92
	2.9	9.89	1.33
	2.4	4.62	0.73
	2.4	5.21	0.79
	2.9	9.71	1.22
Mean	2.6	6.67	0.93
SD	0.2	1.84	0.22

Table 3. Number of Live Clams, *Corbicula fluminea*, Exposed for 28 Days to Test Sediments.

Sample Description	Initial Number					Number at Day 28				
	1	2	3	4	5	1	2	3	4	5
HC-08 (NAS#7577F)	43	43	43	43	43	41	43	43	43	43
HC-10 (NAS#7578F)	43	43	43	43	43	43	43	43	43	43
Control (7579F)	43	43	43	43	43	43	43	43	43	43

APPENDIX I

PROTOCOL

TEST PROTOCOL

FRESHWATER CLAM, *CORBICULA FLUMINEA*, 28-DAY SEDIMENT BIOACCUMULATION

1. INTRODUCTION

1.1 Purpose of Study: Laboratory sediment bioaccumulation tests provide an estimate of contaminant uptake by benthic infauna. The purpose of this study is to expose Asiatic clams (*Corbicula fluminea*) to freshwater sediments for 28 days so that they may bioaccumulate sediment-associated contaminants. After the bioaccumulation period, clam soft tissues are frozen for subsequent tissue analysis.

1.2 Referenced Method: There are currently no standard methods available for testing with this species. The following sources or methods were consulted in development of this protocol: 1) Portland Harbor Sediment Management Plan - Appendix G; 2) EPA/600/R-93/183; 3) EPA 823-B-98-004; 4) and ASTM E-1688-97a.

1.3 Summary of Method: A summary of test conditions for the 28-day clam bioaccumulation test is tabulated on page 6.

The 28-day bioaccumulation test with *Corbicula fluminea* is conducted at 20°C with a 16L:8D photoperiod at an illuminance of about 50-100 ft-c. Test chambers are 5 to10-gallon aquaria containing 3.7 to 7 L of sediment and 10 to 20 L of overlying water. Twenty to forty clams (~1g soft tissue/clam) are used in each of five replicate aquaria to give a stocking density of 20-40 g wet tissue per replicate (≈250g wet sediment per g soft tissue). Test organisms are not fed during the test. Gentle aeration is employed to insure that dissolved oxygen concentration does not fall below 40% saturation. Each chamber receives three repalcement volumes per week of overlying water. Overlying water can be culture water, well water, surface water, site water, reconstituted water, or dechlorinated municipal water. The test endpoint is bioaccumulation.

2. STUDY MANAGEMENT

2.1 Sponsor's Name and Address:
_____
_____
_____
_____

2.2 Sponsor's Study Monitor:
_____

2.3 Name of Testing Laboratory:
Northwestern Aquatic Sciences
3814 Yaquina Bay Road, P.O. Box 1437
Newport, OR 97365.

2.4 Test Location: _____

2.5 Laboratory's Personnel to be Assigned to the Study:
Study Director: _____
Quality Assurance Unit: _____
Aquatic Toxicologist: _____
Aquatic Toxicologist: _____

2.6 Proposed Testing Schedule: The time between sediment collection and use in testing should be kept to a minimum; therefore, bioaccumulation testing is started as soon after sample receipt as logistically possible. For many applications a maximum holding time of 8 weeks is employed.

2.7 Good Laboratory Practices: The test is conducted following the principles of Good Laboratory Practices (GLP) as defined in the EPA/TSCA Good Laboratory Practice regulations revised August 17, 1989 (40 CFR Part 792).

3. TEST MATERIAL

The test materials are freshwater sediments. It is recommended that he control, reference, and test sediments be placed in clean, air-tight containers. Sediments for metals bioaccumulation should be stored in the absence of air to minimize the oxidation of reduced forms. Nitrogen can be used to fill the headspace in the containers. Glass containers are recommended for sediments polluted with either metals or organics, although high-density polyethylene and PTFE containers are also acceptable. Large organisms and extraneous materials, such as bivalves or twigs, should be removed from the sediments before storing. At the laboratory the samples are stored at 4°C in the dark. The time between sediment collection and use in testing should be kept to a minimum. A maximum holding time of eight weeks is recommended. A negative control sediment is collected from a clean site or the animal collection site, and should contain no or very low concentrations of the contaminant(s) of concern. In addition, a reference sediment is normally employed as a comparison station when evaluating dredged materials

4. TEST WATER

Test water (overlying water) at NAS is normally moderately hard synthetic water at a hardness of 80-100 mg/L as $CaCO_3$ and alkalinity of 60-70 mg/L as $CaCO_3$. Dilution water is prepared from dechlorinated and deionized municipal water or Milli-Q reagent grade water and reagent grade chemicals. Test water may also be well water, surface water, site water depending on the study needs

5. TEST ORGANISMS

5.1 Species: clam, *Corbicula fluminea*.

5.2. Source: Commercial suppliers or field collection from uncontaminated sites.

5.3 Age: adult (~1g soft tissue/clam)

5.4 Acclimation and Pretest Observation: After receipt, clams should be held in the laboratory for at least 24 hours prior to test initiation in order to assess their health and acclimate them to test conditions. Mortality during the holding period should not be excessive

6. DESCRIPTION OF TEST SYSTEM

6.1 Test Chambers and Environmental Control: Test chambers generally used in the toxicity test are 5-10 gal aquaria. Test chambers are maintained at constant temperature by partial immersion in a temperature-controlled water bath or by placement in a temperature-controlled room. Gentle aeration is employed to insure that dissolved oxygen concentration does not fall below 40% saturation. The test is conducted under an illuminance of 10-20 $\mu E/m^2/s$ (50-100 ft-c) with a 16L:8D photoperiod

6.2 Cleaning: All laboratory glassware, including test chambers, is cleaned as described in EPA/600/4-90/027F. New glassware and test systems are soaked 15 minutes in tap water and scrubbed with detergent (or cleaned in automatic dishwasher); rinsed twice with tap water; carefully rinsed once with fresh, dilute (10%, V:V) hydrochloric or nitric acid to remove scale, metals, and bases; rinsed twice with deionized water; rinsed once with acetone to remove organic compounds (using a fume hood or canopy); and rinsed three times with deionized water. Test systems and chambers are rinsed again with dilution water just before use.

7. EXPERIMENTAL DESIGN AND TEST PROCEDURES

7.1 Experimental Design: The test involves exposure of clams to test, control, and reference sediments. The sediments are placed on the bottom of the test containers and are overlain with test water. The test exposure is for 28 days. Static replacement of overlying water is performed tree times a week. Each treatment consists of five replicate test containers, each containing approximately 20-40 organisms (20-40 g soft tissue wet weight). Test chamber positions are completely randomized. Test organisms are randomly distributed to the test chambers. Animals are placed on the sediment surface and allowed to bury.

7.2 Setup of Test Containers: Sediments are homogenized and placed in test chambers on the day before addition of test organisms. From 3.5-7 L of sediment is placed into each of five replicate aquaria. After addition of the sediment, 10-20 L of test water is gently added to each test container in a manner to prevent resuspension. The test begins when clams are introduced to the test chambers. Initial water quality measurements are taken prior to the addition of test organisms.

7.3 Test Conditions: Gentle aeration is employed to insure that dissolved oxygen concentration does not fall below 40% saturation. The test temperature employed is $20 \pm 1°C$). A 16:8, L:D photoperiod is used. Illumination is supplied by daylight fluorescent lamps at 10-20 $\mu E/m^2/s$ (50-100 ft-c). The overlying water is replaced three times a week.

7.4 Beginning the Test: The test is begun by adding the organisms to the equilibrated test containers as previously described. A five replicate zero-time sample of test animals is preserved (frozen) for analysis of initial concentrations of chemicals of concern.

7.5 Feeding: None.

7.6 Test Duration, Type and Frequency of Observations, and Methods: The duration of the bioaccumulation test is 28 days. The type and frequency of observations to be made are summarized as follows:

TYPE OF OBSERVATION	TIMES OF OBSERVATION
BIOLOGICAL DATA	
Survival	Daily; any dead animals are removed
PHYSICAL AND CHEMICAL DATA	
Hardness, alkalinity, conductivity, pH and total ammonia	Beginning and end of test in overlying water. One replicate per treatment. (optionally on days 0, 7, 14, 21, 28).
Dissolved oxygen, temperature	Daily in overlying water. One replicate per treatment

Dissolved oxygen is measured using a polarographic oxygen probe calibrated according to the manufacturer's recommendations. The pH is measured using a pH probe and a properly calibrated meter with scale divisions of 0.1 pH units. Temperature is measured with a calibrated mercury thermometer or telethermometer. Conductivity is measured with a conductivity meter. Hardness and alkalinity are measured using titrometric methods. Ammonia-nitrogen is measured using the salicylate colerimetric method (Clin. Chim. Acta 14:403, 1996).

7.7 Test Termination and Depuration: At test termination, animals are removed from the sediment by gently sieving test chamber contents. Any gaping animals that are unresponsive to gentle prodding should be considered dead and excluded from subsequent tissue analysis. All surviving organisms from an individual replicate should be transferred to an aquarium containing clean water for 24 hours to purge their gut contents. After this 24-hour period, whole animals are placed in clean containers and frozen for subsequent tissue residue analysis.

7.8 Criteria of Test Acceptance: The test results are acceptable if the test organisms burrow into the sediments; the test should be considered invalid if overt sediment avoidance is observed.

8. DATA ANALYSIS

The endpoint of the test is bioaccumulation. Surviving clams are depurated for 24 hours and then frozen for subsequent tissue analysis. Data analysis consists of calculating means and standard deviations for tissue chemical concentrations and water quality parameters. Statistical comparisons of treatment groups may be done using standard hypothesis test procedures (i.e. test for normality and homogeneity followed by parametric or non-parmetric comparison tests as appropriate).

9. REPORTING

The final report of the test results must include all of the following standard information at a minimum (except that tabulation and analysis of tissue chemical concentrations is optional depending on client requirements): name and identification of the test; the investigator and laboratory; date and time of test beginning and end; information on the test material; information on the source and quality of the overlying/test water; detailed information about the test organisms including acclimation conditions; a description of the experimental design and test chambers and other test conditions including water quality; definition of the effect criteria and other observations; responses, if any, in the control treatment, tabulation and statistical analysis of measured responses and a summary table of endpoints; a description of the statistical methods used; any unusual information about the test or deviations from procedures; results of the initial screening toxicity test.

10. STUDY DESIGN ALTERATION

Amendments made to the protocol must be approved by the sponsor and study director and should include a description of the change, the reason for the change, the date the change took effect and the dated signatures of the study director and sponsor. Any deviations in the protocol must be described and recorded in the study raw data.

11. REFERENCED AND/OR CONSULTED GUIDELINES

ASTM. 1997. Determination of the Bioaccumulation of Sediment-Associated Contaminants by Benthic Invertebrates. ASTM Standard Method No. E 1688 – 97a. Am. Soc. Test. Mat., Philadelphia, PA.

Portland Harbor Sediment Management Plan. June 25, 1999. Oregon Department of Environmental Quality.

U.S. EPA. 1993. Guidance Manual: Bedded Sediment Bioaccumulation Tests: Bedded Sediment Bioaccumulation Tests. EPA/600/R-93/183.

U.S. EPA. 1998. Evaluation of Dredged Material Proposed for Discharge in Waters of the U.S. – Testing Manual: Inland Testing Manual. EPA 823-B-98-004.

Weber, C.I. (Ed.) 1993. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms (Fourth Edition). EPA/600/4-90/027F.

12. APPROVALS

_____ for _____
Name Date

_____ for Northwestern Aquatic Sciences
Name Date

SUMMARY OF TEST CONDITIONS

***CORBICULA FLUMINEA*,**
28-DAY SEDIMENT BIOACCUMULATION

1. Test type:	Static Renewal
2. Test duration:	28 days
3. Temperature:	20± 1° C
4. Light quality:	Ambient Laboratory
5. Light itensity:	10-20 µE/m^2/s (50-100 ft-c)
6. Photoperiod:	16L:8D
7. Test chamber size:	5 - 10 gal aquarium
8. Sediment volume:	3.5 - 7 L (≈250g wet sediment per g soft tissue)
9. Overlying water volume:	10 - 20 L
10. Renewal of overlying water:	Static Renewal = 3x/wk
11. Age of test organisms:	Adults (~1g soft tissue/clam)
12. Loading of organisms in chamber:	20 - 40 (20-40 g soft tissue wet weight)
13. Replicates per sediment:	5
14. Test chamber cleaning	None
15. Feeding:	None
16. Aeration:	Gentle, as needed
17. Overlying water:	Culture water, well water, surface water, site water, reconstituted water , or dechlorinated municipal water
18. Study treatments:	Site sediment(s), a reference sediment, and a control sediment.
19. Overlying water quality:	Hardness, alkalinity, conductivity, pH, and ammonia at the beginning and end of a test. Temperature and dissolved oxygen daily.
20. Endpoint:	Bioaccumulation
21. Sample holding requirements:	< 8 weeks
22. Sample volume required:	18 - 36 L (5-10 gal)
23. Test acceptability:	1. Adequate mass of test organisms at test completion for detection of target analytes. 2. Test organisms must burrow into the test sediments.

APPENDIX C
CHEMICAL DATA VALIDATION REPORT

APPENDIX C
CHEMICAL DATA VALIDATION REPORT

Sediment and Tissue Analytical Data

The following criteria were evaluated in the standard data quality review process:

- Holding times;
- Method blanks;
- Surrogate recoveries;
- Laboratory control sample/laboratory control sample duplicate (LCS/LCSD) recoveries;
- Matrix spike/matrix spike duplicate (MS/MSD) recoveries; and
- Laboratory and field duplicate relative percent difference (RPD).

K2106275: Sediment Samples (Date Received – August 29, 2001)

Nine sediment samples were submitted to Columbia Analytical Services, Inc., (CAS) laboratory. The following analyses were performed:

- Inorganic Parameters (Total Solids via Method 160.3 and Total Solids, Volatile via Method 160.4M);
- Fuel Identification and Quantitation – Silica Gel Treated (EPA Method 3550B/8015M);
- Organochlorine Pesticides (EPA Method 3540C/8081A); and
- Polychlorinated biphenyls (PCBs, EPA Method 3540/8082).

Inorganic Parameters (Total Solids and Total Solids, Volatile). No anomalies associated with the analysis of these samples were observed.

Fuel Identification and Quantitation – Silica Gel Treated. All required holding times were met. No method blank contamination was detected. Surrogate, LCS and MS recoveries were within laboratory control limits. MS/MSD duplicate RPDs were within laboratory control limits.

Organochlorine Pesticides. All required holding times were met. No method blank contamination was detected. Surrogate, LCS, and MS recoveries were within laboratory control limits. MS/MSD duplicate RPDs were acceptable.

PCBs. All required holding times were met. No method blank contamination was detected. Surrogate, LCS, and MS recoveries were within laboratory control limits. MS/MSD duplicate RPDs were acceptable.

K2106748: Sediment Samples (Date Received – September 17, 2001)

Eight sediment samples were submitted to Columbia Analytical Services, Inc., (CAS) laboratory. The following analyses were performed:

- Inorganic Parameters;
- Total Metals (EPA 6000/7000 Series Methods);
- Organochlorine Pesticides (EPA Method 8081A);
- Polychlorinated biphenyls (PCBs, EPA Method 8082);
- Semivolatile Organic Compounds (SVOCs, EPA Method 8270C); and
- Organotin Compounds (Porewater, Extraction Method Stallard, Analysis Method GC-FPD).

Inorganic Parameters (Total Solids and Total Organic Carbon). No anomalies associated with the analysis of these samples were observed.

Total Metals. All required holding times were met. No method blank contamination was detected. LCS and MS recoveries were within laboratory control limits with the following exceptions. Antimony MS recovery of 40 percent is less than control limits (70 to 130 percent). Associated results were qualified as estimated (J or UJ). The MS recovery for Zinc was –43 percent; however, the zinc sample concentration was more than four times the spike concentration preventing accurate evaluation of the spike recovery. No qualification was necessary based on the zinc MS recovery. The laboratory duplicate RPDs were within laboratory control limits.

Organochlorine Pesticides. All required holding times were met. No method blank contamination was detected. Surrogate, LCS, and MS recoveries were within laboratory control limits. MS/MSD RPDs were acceptable.

PCBs. All required holding times were met. No method blank contamination was detected. Surrogate, LCS, and MS recoveries were within laboratory control limits. MS/MSD RPDs were acceptable.

SVOCs. All required holding times were met. No method blank contamination was detected. Surrogate, LCS, and MS recoveries were within laboratory control limits. MS/MSD RPDs were acceptable.

Organotin Compounds in Porewater. All required holding times were met. No method blank contamination was detected. Surrogate, LCS, and MS recoveries were within laboratory control limits with the following exceptions. The MS recoveries for tetra-n-butyltin and n-butyltin were less than laboratory control limits. All associated sample results for these analytes were nondetect and were flagged as estimated (UJ). The LCS recovery for n-butyltin was less than laboratory control limits and all associated results were flagged as estimated (UJ). MS/MSD RPDs were acceptable.

K2107717: Tissue Samples (Date Received – October 16, 2001)

Five tissue samples were submitted to the CAS laboratory. The following analyses were performed:

- Inorganic Parameters;
- Total Metals (EPA 6000/7000 Series Methods);
- Organochlorine Pesticides (EPA Method 8081A);
- Polychlorinated biphenyls (PCBs, EPA Method 8082);
- Semivolatile Organic Compounds (SVOCs, EPA Method 8270C); and
- Organotins.

Inorganic Parameters (Total Solids). No anomalies associated with the analysis of these samples were observed.

Total Metals. All required holding times were met. Antimony was detected in the initial calibration blank at a concentration of 0.06 µg/kg. Antimony was not detected in any of the continuing calibration or preparation blanks therefore no qualification of the antimony results is necessary. Copper, lead, and zinc were detected in the preparation blank at concentrations of 2.6, 0.02, and 0.2 mg/kg, respectively. Lead and zinc were detected in the associated samples at concentrations greater than five times that detected in the preparation blank, therefore, the sample results for these metals were not qualified. Copper results

for four of the five tissue samples were greater than the method reporting limit (MRL), but less than five times the preparation blank copper concentration. The copper results for these samples were qualified as non-detects with the MRLs elevated to the copper concentrations found in each sample. The copper result of 13.5 mg/kg for the fifth tissue sample (7651F) is slightly greater than five times the copper preparation blank concentration (13 mg/kg), and no qualification of this sample result based on blank contamination was necessary. LCS and MS recoveries, laboratory duplicate RPDs, ICP interference check sample results, and ICP serial dilution results were within laboratory control limits.

Organochlorine Pesticides. All required holding times were met. No method blank contamination was detected. Surrogate, LCS, and MS recoveries were within laboratory control limits with the following exceptions. The surrogate recoveries for all samples were slightly below laboratory control limits (50 to 71 percent versus 70 to 130 percent). According to the CAS Case Narrative, these laboratory control limits are the default SVOC limits since CAS has not determined control limits for pesticides in tissue. Because the surrogate recoveries were consistent between the five samples and were not grossly below the laboratory control limits used, the associated sample results were not qualified. The LCS/LCS Dup RPDs as well as the Initial Calibration and Seondary Source Calibration Verification results were acceptable.

PCBs. All required holding times were met. No method blank contamination was detected. Surrogate, LCS, and MS recoveries were within laboratory control limits. LCS/LCSD RPDs were acceptable. No MS/MSD was run due to limited sample volume. The LCS/LCSD were used as a substitute.

SVOCs. All required holding times were met. Phenol was detected in the method blank at a concentration of 73 µg/kg. All associated phenol sample concentrations were greater than five times the blank concentration and were, therefore, not qualified. Surrogate, LCS, and MS recoveries were within laboratory control limits with the following exceptions. Two of the acid fraction surrogates exceeded laboratory control limits in samples 7647F and 7650F. All associated detected analytes were flagged as estimated (J). The surrogate results for all other samples were acceptable. The LCS/LCSD RPD for benzoic acid was greater than laboratory control limits (67 percent versus 40 percent). However, benzoic acid was not detected in any of the tissue samples and the LCS/LCS percent recoveries were acceptable. The benzoic acid sample results were not qualified.

Butyltins. All required holding times were met. No method blank contamination was detected. Surrogate and LCS recoveries were within laboratory control limits with the following exceptions. The surrogate recovery

for sample 7648F was greater than laboratory control limits (127 percent versus 22 to 119 percent). All associated detections were qualified as estimated (J). The LCS/LCSD RPDs were acceptable.

K2108269: Tissue Samples (Date Received – November 7, 2001)

Twenty tissue samples were submitted to the CAS laboratory. The following analyses were performed:

- Inorganic Parameters;
- Total Metals (EPA 6000/7000 Series Methods);
- Butyltin Compounds (Extraction Method Stallard, Analysis Method GC-FPD);
- Lipids (Preparation – EPA Method 3540);
- Organochlorine Pesticides (EPA Method 8081A);
- Polychlorinated biphenyls (PCBs, EPA Method 8082);
- Semivolatile Organic Compounds (SVOCs, EPA Method 8270C); and
- Organotins.

Inorganic Parameters (Total Solids). No anomalies associated with the analysis of these samples were observed.

Total Metals. All required holding times were met. Antimony, copper, and zinc were detected at low levels in the initial calibration blank and/or one of the continuing calibration blanks. These metals were either not detected in site samples or were detected at concentrations greater than five times that detected in the calibration blanks. No qualification was necessary. Copper, lead, mercury, and zinc were detected in the method blank at concentrations of 0.05, 0.04, 0.02, and 0.34 mg/kg. All four metals were detected in the associated samples at concentrations greater than five times that detected in the method blank, therefore, the sample results for these four metals were not qualified. LCS and MS recoveries, laboratory duplicate RPDs, and ICP interference check results were within laboratory control limits with the following exception. The MS chromium recovery exceeded the laboratory control limits. Associated chromium sample results were flagged as estimated (J).

Butyltins. All required holding times were met. No method blank contamination was detected. Surrogate and LCS recoveries were within laboratory control limits. The LCS/LCSD RPDs were acceptable. The confirmation comparisons were acceptable. The tetrabutyltin continuing

calibration verification results associated with samples 7659F and 7661F were slightly elevated on both columns. Tetrabutyltin was not detected in either sample and was qualified as estimated (UJ).

Lipids. No anomalies associated with the analysis of these samples were observed.

Organochlorine Pesticides. All required holding times were met. No method blank contamination was detected. Surrogate, LCS, and MS recoveries were within laboratory control limits with the following exceptions. The LCS recovery for 4,4'-DDT was greater than laboratory control limits, while the LCSD recovery and the LCS/LCS Dup RPD were acceptable. 4,4'-DDT was not detected in any of the associated samples and did not, therefore, require qualification. The Secondary source verification and the continuing calibration verification results were acceptable as discussed in the Case Narrative. The confirmation comparison results for 4,4'-DDE, gamma-chlordane, and alpha-chlordane were greater than laboratory control limits in numerous samples. Sample results that were greater than the MRL were qualified as estimated (J), while no additional qualification was required for the other samples with results less than the MRL.

PCBs. All required holding times were met. No method blank contamination was detected. Surrogate, LCS, and MS recoveries were within laboratory control limits. LCS/LCSD RPDs were acceptable. No MS/MSD was run due to limited sample volume. The LCS/LCSD were used as a substitute. All confirmation comparison results were less than laboratory control limits.

SVOCs. All required holding times were met. Pyrene, dibenz(a,h)anthracene, and benzo(g,h,I)perylene were detected in the method blank at a concentrations of 2.4 J, 3.3 J, and 2.6 J µg/kg, respectively. These three compounds were either not detected or were detected at concentrations greater than five times the blank concentration, with the following exceptions:

- Sample 7657F. Initial pyrene result of 7.0 J changed to 40 U;
- Sample 7658F. Initial pyrene result of 5.8 J changed to 40 U;
- Sample 7659F. Initial pyrene result of 5.5 J changed to 40 U; and
- Sample 7717F. Initial pyrene result of 10 J changed to 40 U.

Surrogate, LCS, and MS recoveries were within laboratory control limits with the following exceptions. One base/neutral and/or one acid fraction surrogates exceeded control limits in all samples. No qualification is necessary when only one surrogate is out of compliance. The internal standard recovery of perylene-

d12 was below control limits in samples 7557F, 7659F, 7661F, and 7660F. According to the Case Narrative, results quantified using perylene-d12 may include a high bias due to potential matrix effects. No associated analytes were detected above their respective MRLs. All associated analytes were qualified as estimated (J or UJ). The LCS/LCSD RPDs were within control limits. The initial calibration and secondary source verification results were acceptable as discussed in the Case Narrative.

K2108883: Tissue Samples (Date Received – November 29, 2001)

Fifteen tissue samples were submitted to the CAS laboratory. The following analyses were performed:

- Inorganic Parameters;
- Total Metals (EPA 6000/7000 Series Methods);
- Butyltin Compounds (Extraction Method Stallard, Analysis Method GC-FPD);
- Lipids (Preparation – EPA Method 3540);
- Organochlorine Pesticides (EPA Method 8081A);
- Polychlorinated biphenyls (PCBs, EPA Method 8082);
- Semivolatile Organic Compounds (SVOCs, EPA Method 8270C); and
- Organotins

Inorganic Parameters (Total Solids). No anomalies associated with the analysis of these samples were observed.

Total Metals. All required holding times were met. Antimony was detected in the initial calibration blank and in the continuing calibration blanks at concentrations of 0.06 or 0.07 µg/kg. Antimony was not detected in any of the associated samples and did not require qualification. Lead was detected in the method blank at a concentration of 0.01 J mg/kg. However, lead was detected in the associated samples at concentrations greater than five times that detected in the method blank, therefore, the sample results for lead were not qualified. LCS and MS recoveries, laboratory duplicate RPDs, and ICP serial dilution results were within laboratory control limits with the following exception. The LCS lead recovery (1.3 mg/kg) greatly exceeded the laboratory control limit of 0.16 to 0.29 mg/kg. Associated lead sample results were flagged as estimated (J).

Butyltins. All required holding times were met. No method blank contamination was detected. Surrogate and LCS recoveries were within laboratory control limits. The LCS/LCSD RPDs were acceptable. The confirmation comparison of n-butyltin in sample 7716F (71.6 percent) exceeded laboratory control limits of 40 percent. N-butyltine was, therefore, qualified as estimated (J) in sample 7716F.

Lipids. No anomalies associated with the analysis of these samples were observed.

Organochlorine Pesticides. All required holding times were met. No method blank contamination was detected. Surrogate, LCS, and MS recoveries were within laboratory control limits with the following exceptions. The LCS recovery for 4,4'-DDT was greater than laboratory control limits, while the LCSD recovery and the LCS/LCS Dup RPD were acceptable. 4,4'-DDT was not detected in any of the associated samples and did not, therefore, require qualification. The Secondary source verification and the continuing calibration verification results were acceptable as discussed in the Case Narrative. The confirmation comparison results for 4,4'-DDE in samples 7709F, 7713F, and 7714F and gamma chlordane in sample 7717F were greater than laboratory control limits. However, all sample results, with the exception of 4,4'-DDE in sample 7713F, were less than MRLs. Therefore, 4,4'-DDE in sample 7713 was qualified as estimated (J), while no additional qualification was required for the other samples and gamma chlordane.

PCBs. All required holding times were met. No method blank contamination was detected. Surrogate, LCS, and MS recoveries were within laboratory control limits. LCS/LCSD RPDs were acceptable. No MS/MSD was run due to limited sample volume. The LCS/LCSD were used as a substitute. All confirmation comparison results were less than laboratory control limits.

SVOCs. All required holding times were met. Pyrene, dibenz(a,h)anthracene, and benzo(g,h,I)perylene were detected in the method blank at a concentrations of 2.4 J, 3.3 J, and 2.6 J µg/kg, respectively. These three compounds were either not detected or were detected at concentrations greater than five times the blank concentration, with the following exceptions:

- Sample 7711F. Initial benzo(g,h,I)perylene result of 4.5 J changed to 73 UJ (J qualifier based on internal standard issue discussed below);
- Sample 7713F Initial pyrene result of 7.7 J changed to 80 U;
- Sample 7716F Initial pyrene result of 8.4 J changed to 54 U; and
- Sample 7717F Initial pyrene result of 9.9 J changed to 73 U.

Surrogate, LCS, and MS recoveries were within laboratory control limits with the following exceptions. Two base/neutral and one acid fraction surrogates exceeded control limits in all samples. All base/neutral detections were flagged as estimated (J). One acid fraction surrogate exceeded its control limit in the method blank. No qualification was necessary since only one surrogate was out of compliance. The internal standard recovery of perylene-d12 was below control limits on January 3, 2001. According to the Case Narrative, results quantified using perylene-d12 may include a high bias due to potential matrix effects. No associated analytes were detected above their respective MRLs. All associated analytes were qualified as estimated (J or UJ). The LCS/LCSD RPDs were within control limits. The initial calibration and secondary source verification results were acceptable as discussed in the Case Narrative.